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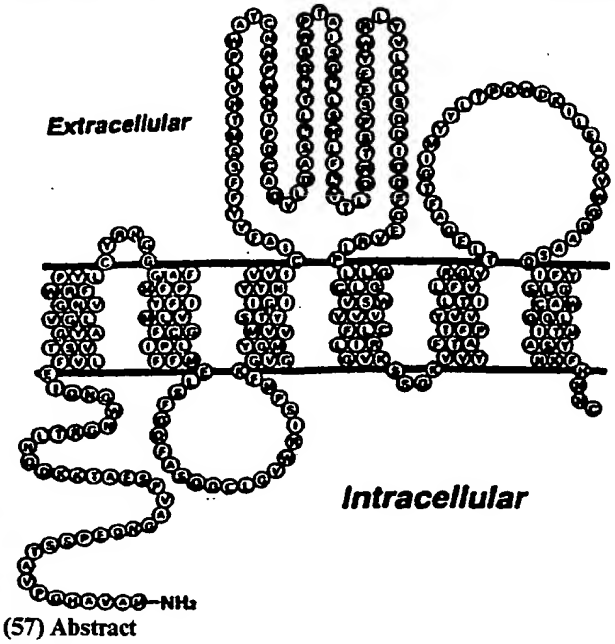

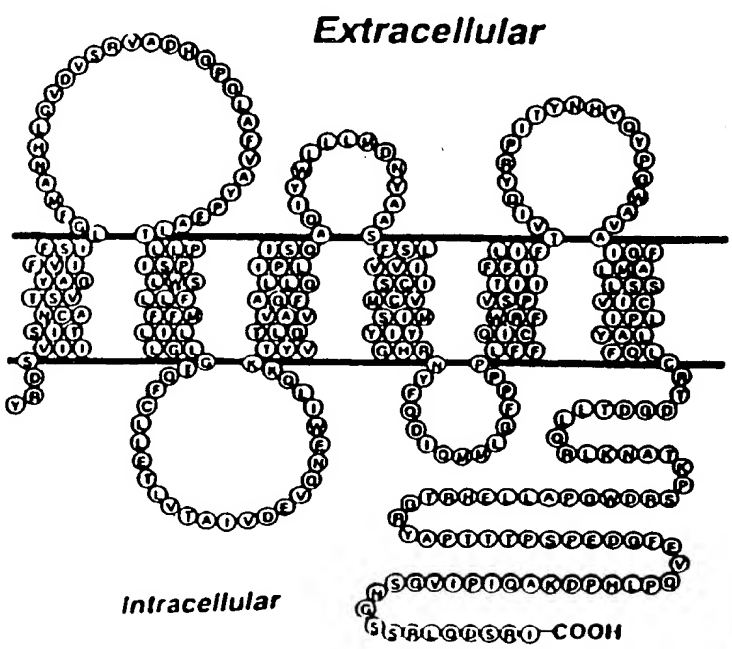

(57) Abstract

This invention provides isolated nucleic acid molecules encoding a mammalian or human glycine transporter, vectors comprising the isolated nucleic acid molecules, mammalian cells comprising such vectors, nucleic acid probes, antisense oligonucleotides complementary to any sequence of a nucleic acid molecule which encodes a mammalian glycine transporter, and non-human transgenic animals which express DNA encoding a normal or a mutant mammalian glycine transporter. The invention also provides the mammalian or human glycine transporter proteins, antibodies directed to them, and pharmaceutical compounds related to the human glycine transporter. The invention further provides methods for determining ligand binding, detecting expression, drug screening, as well as treatments for alleviating abnormalities associated with mammalian or human glycine transporters.

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DNA ENCODING A GLYCINE TRANSPORTER AND USES THEREOF

Background of the Invention

This application is a continuation-in-part of U.S. Serial No. 791,927, filed November 12, 1991, the contents of which are incorporated by reference into the present disclosure.

Throughout this application various publications are referred to by partial citations within parenthesis. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications, in their entireties, are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

An essential property of synaptic transmission is the rapid termination of action following neurotransmitter release. For many neurotransmitters including catecholamines, serotonin, and certain amino acids (e.g., gamma-aminobutyric acid (GABA), glutamate, and glycine), rapid termination of synaptic action is achieved by the uptake of the transmitter into the presynaptic terminal and surrounding glial cells (Bennett et al., 1974; Horn, 1990; Kanner and Schuldiner, 1987). Inhibition or stimulation of neurotransmitter uptake provides a means for modulating the strength of the synaptic action by regulating the available levels of endogenous transmitters. The development of selective inhibitors may therefore represent a novel therapeutic approach to the

treatment of neurological disorders.

The amino acid glycine is an important neurotransmitter in the vertebrate central nervous system, where it serves two distinct functions. First, glycine is a classical inhibitory neurotransmitter with a well established role in the spinal cord, brainstem, and retina (Aprison, 1990; Daly, 1990; Cortes and Palacios, 1990). The inhibitory effects of glycine are mediated by the glycine receptor, a ligand-gated chloride channel which is activated by glycine and competitively antagonized by strychnine (Grenningloh et al., 1987). Blockade of glycinergic transmission by strychnine causes seizures in animals and humans. Thus, agents which enhance the inhibitory role of glycine in the CNS may ameliorate the symptoms of epilepsy or other neurological disorders associated with excessive neural and/or musculoskeletal activity. This hypothesis is supported by the finding that defects in the glycine receptor underlie the hereditary myoclonus observed in certain mutant strains of mice (Becker, 1990) and calves (Gundlach, 1990).

In addition to its inhibitory role, glycine also modulates excitatory neurotransmission by potentiating the action of glutamate at NMDA receptors, both in hippocampus and elsewhere (Johnson and Ascher, 1987; for review, see Fletcher et al., 1990). The glycine regulatory site on the NMDA receptor is distinct from the strychnine-sensitive glycine receptor (Fletcher et al., 1990). The NMDA class of glutamate receptors is known to play a critical role in long-term potentiation, a cellular model of learning (Collingridge and Bliss, 1987). Recent evidence suggests that activation of the glycine regulatory site on the NMDA receptor may enhance cognitive function (Handelmann et al., 1989).

The molecular properties of glycine transport, particularly in relation to the dual role of glycine in the nervous system, have not previously been studied. Elucidation of the molecular structure of the synaptic glycine transporter is an important step in understanding glycinergic transmission and modulation. In particular, we were interested in exploring whether separate transporter mRNAs encode the uptake proteins that regulate inhibitory transmission and those that modulate glutamatergic transmission or whether one transporter mediates both functions.

Summary of the Invention

This invention provides an isolated nucleic acid molecule encoding a mammalian glycine transporter. In one embodiment of this invention, the nucleic acid molecule comprises a plasmid designated pSVL-rB20a (ATCC Accession No. 75132). In the preferred embodiment this invention provides an isolated nucleic acid molecule encoding a human glycine transporter. In one embodiment of this invention, the nucleic acid molecule comprises a plasmid designated pBluescript-hTC27a (ATCC Accession No.).

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a mammalian glycine transporter. This invention also provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human glycine transporter.

This invention provides an antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a mammalian glycine transporter so as to prevent translation of the mRNA molecule. This invention further provides an antisense oligonucleotide having a sequence capable of binding specifically to an mRNA molecule encoding a human glycine transporter so as to prevent translation of the mRNA molecule.

A monoclonal antibody directed to a mammalian glycine transporter is also provided by this invention. A monoclonal antibody directed to a human glycine

transporter is further provided by this invention.

This invention provides a pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a mammalian glycine transporter and a pharmaceutically acceptable carrier as well as a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of glycine transporter and a pharmaceutically acceptable carrier.

This invention further provides a pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a mammalian glycine transporter and a pharmaceutically acceptable carrier as well as a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of glycine transporter and a pharmaceutically acceptable carrier.

This invention provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a mammalian glycine transporter so placed positioned within such genome as to be transcribed into antisense mRNA complementary to mRNA encoding the glycine transporter and when hybridized to mRNA encoding the glycine transporter, the complementary mRNA reduces the translation of the mRNA encoding the glycine transporter.

This invention provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a human glycine transporter so placed positioned within such genome as to be transcribed into antisense mRNA complementary to mRNA encoding the glycine transporter and when hybridized to

mRNA encoding the glycine transporter, the complementary mRNA reduces the translation of the mRNA encoding the glycine transporter.

This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a mammalian glycine transporter so placed positioned within such genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the transporter and when hybridized to mRNA encoding the transporter, the antisense mRNA thereby prevents the translation of mRNA encoding the transporter.

This invention also provides a transgenic, nonhuman mammal whose genome comprises DNA encoding a human glycine transporter so placed positioned within such genome as to be transcribed into antisense mRNA which is complementary to mRNA encoding the transporter and when hybridized to mRNA encoding the transporter, the antisense mRNA thereby prevents the translation of mRNA encoding the transporter.

This invention provides a method of screening drugs to identify drugs which specifically interact with, and bind to, a mammalian glycine transporter on the surface of a cell which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding a mammalian glycine transporter, the protein encoded thereby is expressed on the cell surface, with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, a mammalian glycine transporter.

This invention provides a method of screening drugs to identify drugs which specifically interact with, and bind to, a human glycine transporter on the surface of a cell which comprises contacting a mammalian cell comprising an

isolated DNA molecule encoding a human glycine transporter, the protein encoded thereby is expressed on the cell surface, with a plurality of drugs, determining those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, a human glycine transporter.

This invention also provides a method of determining the physiological effects of expressing varying levels of mammalian glycine transporters which comprises producing a transgenic nonhuman animal whose levels of mammalian glycine transporter expression are varied by use of an inducible promoter which regulates mammalian glycine transporter expression.

This invention also provides a method of determining the physiological effects of expressing varying levels of human glycine transporters which comprises producing a transgenic nonhuman animal whose levels of human glycine transporter expression are varied by use of an inducible promoter which regulates human glycine transporter expression.

This invention further provides a method of determining the physiological effects of expressing varying levels of mammalian glycine transporters which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of mammalian glycine transporter.

This invention further provides a method of determining the physiological effects of expressing varying levels of human glycine transporters which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human glycine transporter.

This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a specific mammalian glycine transporter allele which comprises: a.) obtaining DNA of subjects suffering from the disorder; b.) performing a restriction digest of the DNA with a panel of restriction enzymes; c.) electrophoretically separating the resulting DNA fragments on a sizing gel; d.) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a mammalian glycine transporter and labelled with a detectable marker; e.) detecting labelled bands which have hybridized to the DNA encoding a mammalian glycine transporter labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f.) preparing DNA obtained for diagnosis by steps a-e; and g.) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a specific human glycine transporter allele which comprises: a.) obtaining DNA of subjects suffering from the disorder; b.) performing a restriction digest of the DNA with a panel of restriction enzymes; c.) electrophoretically separating the resulting DNA fragments on a sizing gel; d.) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human glycine transporter and labelled with a detectable marker; e.) detecting labelled bands which have hybridized to the DNA encoding a human glycine

transporter labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f.) preparing DNA obtained for diagnosis by steps a-e; and g.) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

This invention provides a method for determining whether a substrate not known to be capable of binding to a mammalian glycine transporter can bind to the mammalian glycine transporter which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding the mammalian glycine transporter with the substrate under conditions permitting binding of substrates known to bind to a transporter, detecting the presence of any of the substrate bound to the glycine transporter, and thereby determining whether the substrate binds to the mammalian glycine transporter.

This invention provides a method for determining whether a substrate not known to be capable of binding to a human glycine transporter can bind to the mammalian glycine transporter which comprises contacting a mammalian cell comprising an isolated DNA molecule encoding the human glycine transporter with the substrate under conditions permitting binding of substrates known to bind to a transporter, detecting the presence of any of the substrate bound to the glycine transporter, and thereby determining whether the substrate binds to the human glycine transporter.

Brief Description of the Figures

Figure 1. Nucleotide Sequence, Deduced Amino Acid Sequence and Putative Membrane Topology of the Rat Glycine Transporter. (A). Nucleotides are presented in the 5' to 3' orientation and the coding region is numbered starting from the putative initiating methionine and ending in the termination codon. DNA sequence was determined by the chain termination method of Sanger (1977) on denatured double-stranded plasmid templates using Sequenase. (B). Deduced amino acid sequence (designated by single letter abbreviation) by translation of a long open reading frame is shown. The transporter has been modeled with a similar topology to the previously cloned GABA transporter GAT-1 (Guastella et al., 1990). Postulated N-linked glycosylation sites are shaded.

Figure 2. Comparison of the rat glycine transporter with the human norepinephrine transporter and the rat GABA transporter. The twelve putative α-helical membrane spanning domains (I-XII) are indicated by brackets. Identical residues are shaded. Glycine is the rat glycine transporter; Gaba is the rat GABA transporter (GAT-1); Norepi is the human norepinephrine transporter.

Figure 3. Glycine transport by COS cells transfected with clone rB20a. Non-transfected COS cells (control) or COS cells transfected with rB20a were incubated for 10 minutes with 50nM [^{3}H]glycine (sp. act.45Ci/mmole) in either HBS (containing 150mM NaCl) or in a similar solution in which Na^+ was replaced by equimolar Li (Na^+-free), or Cl^- was replaced by acetate (except for calcium chloride, which was replaced by calcium gluconate; Cl^--free). Data show the specific uptake of glycine, expressed as cpm per mg cellular protein (mean ± S.D. of triplicate

determinations). Data are from a single experiment which was repeated with similar results.

Figure 4. Kinetic properties of the cloned glycine transporter. (A). Time-course of glycine transport. COS cells transfected with rB20a were incubated with 50nM [^{3}H]glycine for the indicated times and the accumulated radioactivity was determined. Specific uptake is expressed as pmoles per mg cellular protein; data are from a single experiment that was repeated with similar results. (B). Concentration-dependence of glycine transport. COS cells transfected with rB20a cells were incubated with the indicated concentrations of [^{3}H]glycine for 30 seconds and the accumulated radioactivity was determined. The specific activity of the [^{3}H]glycine was reduced with unlabeled glycine. Data represent specific transport expressed as nmoles per mg cellular protein, and are from a single experiment that was repeated with similar results.

Figure 5. Northern blot analysis of glycine transporter mRNA.

Total RNA (30μg/lane) isolated from various rat brain regions and peripheral tissues was separated on formaldehyde/agarose gels, blotted, and hybridized with ^{32}P-labeled glycine transporter cDNA. The autoradiogram was developed after a six day exposure. Size standards are indicated at the left in kilobases. The hybridizing transcript is $\approx$ 3.8kb. RNA levels were normalized by reprobing the blot with a cDNA probe, designated p1B15, against cyclophilin. Similar results were obtained by using a probe to ß-actin. Quantitation of the RNA blot was performed by densitometer scanning.

Figure 6. In situ hybridization of glycine transporter

mRNA in rat brain. A) Coronal sections of rat brain were hybridized with an ^{35}S-labeled oligonucleotide probe complementary to the 3' untranslated region of the glycine transporter mRNA and exposed to X-OMAT film for 4 days. Note prominent labeling of the dentate gyrus and areas CA1, CA2, and CA3 of the hippocampal formation. B) Parallel sections hybridized with the sense oligonucleotide showed insignificant labeling. No labeling was detected in sections pretreated with RNase A.

Figure 7. Nucleotide Sequence and Deduced Amino Acid Sequence of the Human Glycine Transporter. Nucleotides are presented in the 5' to 3' orientation and the coding region is numbered starting from the putative initiating methionine. DNA sequence was determined by the chain termination method of Sanger (1977) on denatured double-stranded plasmid templates using Sequenase. Deduced amino acid sequence (single letter abbreviation) by translation of a long open reading frame is shown.

Detailed Description of the Invention

This invention provides an isolated nucleic acid molecule encoding a mammalian glycine transporter. This invention further provides an isolated nucleic acid molecule encoding a human glycine transporter. As used herein, the term "isolated nucleic acid molecule" means a non-naturally occurring nucleic acid molecule that is, a molecule in a form which does not occur in nature. Examples of such an isolated nucleic acid molecule are an RNA, cDNA, or isolated genomic DNA molecule encoding a mammalian glycine transporter and RNA, cDNA or genomic DNA encoding a human glycine transporter. As used herein, "glycine transporter" means a molecule which, under physiologic conditions, is substantially specific for the neurotransmitter glycine, is saturable, and of high affinity for glycine ($K_m \approx 100uM$), and is time and ion dependent. One embodiment of this invention is an isolated nucleic acid molecule encoding a mammalian glycine transporter. Such a molecule may have coding sequences substantially the same as the coding sequence shown in Figure 1. (Sequence I.D. No. 1). The DNA molecule of Figure 1 encodes the sequence of the mammalian glycine transporter gene. Another, preferred embodiment is an isolated nucleic acid molecule encoding a human glycine transporter. Such a molecule may have coding sequences substantially the same as the coding sequence shown in Figure 7. (Sequence I.D. Nos. 5 and 6). The DNA molecule of Figure 7 (Sequence I.D. Nos. 5 and 6) encodes the sequence of the human glycine transporter gene. One means of isolating a mammalian glycine transporter is to probe a mammalian genomic DNA library with a natural or artificially designed DNA probe, using methods well known in the art. Another means of isolating a mammalian

glycine transporter is to probe a mammalian cDNA library with natural or artificially designed DNA, using methods well known in the art. In the preferred embodiment of this invention, the mammalian glycine transporter is a human protein and the nucleic acid molecule encoding a human glycine transporter is isolated from a human cDNA library. In another embodiment of this invention the nucleic acid molcule encoding a human glycine transporter is isolated from a human genomic DNA library. DNA probes derived from the rat glycine transporter gene rB20a are useful probes for this purpose. DNA and cDNA molecules which encode mammalian glycine transporters are used to obtain complementary genomic DNA, cDNA or RNA from human, mammalian or other animal sources, or to isolate related cDNA or genomic clones by the screening of cDNA or genomic libraries, by methods described in more detail below. Transcriptional regulatory elements from the 5' untranslated region of the isolated clone, and other stability, processing, transcription, translation, and tissue specificity determining regions from the 3' and 5' untranslated regions of the isolated gene are thereby obtained.

This invention provides an isolated nucleic acid molecule which has a nucleic acid sequence which differs from the sequence of a nucleic acid molecule encoding a glycine transporter at one or more nucleotides and which does not encode a protein having glycine transporter activity. As used herein "glycine transporter activity" means the ability of the protein to transport glycine. An example of such nucleic acid molecule is an isolated nucleic acid molecule which has an in-frame stop codon inserted into the coding sequence such that the transcribed RNA is not translated into protein.

This invention provides a cDNA molecule encoding a mammalian glycine transporter, wherein the cDNA molecule has a coding sequence substantially the same as the coding sequence shown in Figure 1. (Sequence I.D. No. 1). This invention further provides a cDNA molecule encoding a human glycine transporter, wherein the cDNA molecule has a coding sequence substantially the same as the coding sequence shown in Figure 7. (Sequence I.D. Nos. 5 and 6). These molecules and their equivalents were obtained by the means described above.

This invention also provides an isolated protein which is a mammalian glycine transporter. In one embodiment of this invention, the protein is a mammalian glycine transporter protein having an amino acid sequence substantially similar to the amino acid sequence shown in Figure 1 (Sequence I.D. Nos. 3 and 4). In the preferred embodiment of this invention, the protein is a human glycine transporter protein having an amino acid sequence substantially similar to the amino acid sequence shown in Figure 7. (Sequence I.D. Nos. 5 and 6). As used herein, the term "isolated protein" is intended to encompass a protein molecule free of other cellular components. One means for obtaining isolated glycine transporter is to express DNA encoding the transporter in a suitable host, such as a bacterial, yeast, or mammalian cell, using methods well known to those skilled in the art, and recovering the transporter protein after it has been expressed in such a host, again using methods well known in the art. The transporter may also be isolated from cells which express it, in particular from cells which have been transfected with the expression vectors described below in more detail.

This invention also provides a vector comprising an

isolated nucleic acid molecule such as DNA, RNA, or cDNA, encoding a mammalian glycine transporter. This invention also provides a vector comprising an isolated nucleic acid molecule such as DNA, RNA, or cDNA, encoding a human glycine transporter. Examples of vectors are viruses such as bacteriophages (such as phage lambda), cosmids, plasmids (such as pUC18, available from Pharmacia, Piscataway, NJ), and other recombination vectors. Nucleic acid molecules are inserted into vector genomes by methods well known to those skilled in the art. A specific example of such plasmid is a plasmid comprising cDNA having a coding sequence substantially the same as the coding sequence shown in Figure 1 and designated clone pSVL-rB20a and deposited under ATCC Accession No. 75132. Another example of such plasmid is a plasmid comprising cDNA encoding a human glycine transporter having a coding sequence substantially the same as the coding sequence shown in Figure 7. Alternatively, to obtain these vectors, insert and vector DNA can both be exposed to a restriction enzyme to create complementary ends on both molecules which base pair with each other and are then ligated together with a ligase. Alternatively, linkers can be ligated to the insert DNA which correspond to a restriction site in the vector DNA, which is then digested with the restriction enzyme which cuts at that site. Other means are also available.

This invention also provides vectors comprising a DNA molecule encoding a mammalian glycine transporter, adapted for expression in a bacterial cell, a yeast cell, or a mammalian cell which additionally comprise the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cells so located relative to the DNA encoding a mammalian glycine transporter as to permit expression thereof. DNA having coding sequences

substantially the same as the coding sequence shown in Figure 1 may usefully be inserted into the vectors to express mammalian glycine transporters. This invention also provides vectors comprising a DNA molecule encoding a human glycine transporter, adapted for expression in a bacterial cell, a yeast cell, or a mammalian cell which additionally comprise the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cells so located relative to the DNA encoding a human glycine transporter as to permit expression thereof. DNA having coding sequences substantially the same as the coding sequence shown in Figure 7 may usefully be inserted into the vectors to express human glycine transporters. Regulatory elements required for expression include promoter sequences to bind RNA polymerase and transcription initiation sequences for ribosome binding. For example, a bacterial expression vector includes a promoter such as the lac promoter and for transcription initiation the Shine-Dalgarno sequence and the start codon AUG (Maniatis, et al., Molecular Cloning, Cold Spring Harbor Laboratory, 1982). Similarly, a eukaryotic expression vector includes a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained commercially or assembled from the sequences described by methods well known in the art, for example the methods described above for constructing vectors in general. Expression vectors are useful to produce cells that express the transporter. Certain uses for such cells are described in more detail below.

In one embodiment of this invention a plasmid is adapted for expression in a bacterial, yeast, or, in particular, a mammalian cell wherein the plasmid comprises a DNA

molecule encoding a mammalian glycine transporter and the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cell so located relative to the DNA encoding a mammalian glycine transporter as to permit expression thereof. In another embodiment of this invention a plasmid is adapted for expression in a bacterial, yeast, or, in particular, a mammalian cell wherein the plasmid comprises a DNA molecule encoding a human glycine transporter and the regulatory elements necessary for expression of the DNA in the bacterial, yeast, or mammalian cell so located relative to the DNA encoding a human glycine transporter as to permit expression thereof. Suitable plasmids may include, but are not limited to plasmids adapted for expression in a mammalian cell, e.g., pSVL, pcEXV-3. A specific example of such a plasmid adapted for expression in a mammalian cell is a plasmid comprising cDNA having coding sequences substantially the same as the coding sequence shown in Figure 1 and the regulatory elements necessary for expression of the DNA in the mammalian cell. This plasmid has been designated pSVL-rB20a and deposited under ATCC Accession No. 75132. A preferred embodiment of such a plasmid adapted for expression in a mammalian cell is a plasmid comprising cDNA encoding a human glycine transporter having coding sequences substantially the same as the coding sequence shown in Figure 7 and the regulatory elements necessary for expression of the DNA in the mammalian cell. This plasmid has been designated pBluescript-hTC27a and deposited under ATCC Accession No. . Those skilled in the art will readily appreciate that numerous plasmids adapted for expression in a mammalian cell which comprise DNA encoding mammalian glycine transporters or a human glycine transporters and the regulatory elements necessary to express such DNA in the mammalian cell may be constructed utilizing existing

plasmids and adapted as appropriate to contain the regulatory elements necessary to express the DNA in the mammalian cell. The plasmids may be constructed by the methods described above for expression vectors and vectors in general, and by other methods well known in the art.

The deposit discussed supra was made pursuant to, and in satisfaction of, the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852.

This invention provides a mammalian cell comprising a DNA molecule encoding a mammalian glycine transporter, such as a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, which comprises a DNA molecule encoding a mammalian glycine transporter and the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding a mammalian glycine transporter as to permit expression thereof. This invention provides a mammalian cell comprising a DNA molecule encoding a human glycine transporter, such as a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, which comprises a DNA molecule encoding a human glycine transporter and the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding a human glycine transporter as to permit expression thereof. Numerous mammalian cells may be used as hosts, including, but not limited to, the mouse fibroblast cell NIH3T3, CHO cells, HeLa cells, Ltk^- cells, Cos cells, etc. Expression plasmids such as that described supra may be used to transfect mammalian cells by methods well known in the art such as calcium phosphate

precipitation, or DNA encoding these glycine transporters may be otherwise introduced into mammalian cells, e.g., by microinjection or electroporation, to obtain mammalian cells which comprise DNA, e.g., cDNA or a plasmid, encoding a mammalian glycine transporter or a human glycine transporter.

This invention provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a mammalian glycine transporter, for example with a coding sequence included within the sequence shown in Figure 1. This invention further provides a nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human glycine transporter, for example with a coding sequence included within the sequence shown in Figure 7. As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. Detection of nucleic acid encoding mammalian or human glycine transporters is useful as a diagnostic test for any disease process in which levels of expression of the corresponding glycine transporter are altered. DNA probe molecules are produced by insertion of a DNA molecule which encodes a mammalian or human glycine transporter or fragments thereof into

suitable vectors, such as plasmids or bacteriophages, followed by insertion into suitable bacterial host cells and replication and harvesting of the DNA probes, all using methods well known in the art. For example, the DNA may be extracted from a cell lysate using phenol and ethanol, digested with restriction enzymes corresponding to the insertion sites of the DNA into the vector (discussed above), electrophoresed, and cut out of the resulting gel. An example of such a DNA molecule is shown in Figure 1 and Figure 7. The probes are useful for 'in situ' hybridization or in order to locate tissues which express this gene family, or for other hybridization assays for the presence of these genes or their mRNA in various biological tissues. In addition, synthesized oligonucleotides (produced by a DNA synthesizer) complementary to the sequence of a DNA molecule which encodes a mammalian or a human transporter are useful as probes for these genes, for their associated mRNA, or for the isolation of related genes by homology screening of genomic or cDNA libraries, or by the use of amplification techniques such as the Polymerase Chain Reaction.

This invention also provides a method of detecting expression of a glycine transporter on the surface of a cell by detecting the presence of mRNA coding for a glycine transporter. This method comprises obtaining total mRNA from the cell using methods well known in the art and contacting the mRNA so obtained with a nucleic acid probe as described hereinabove, under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the glycine transporter by the cell. Hybridization of probes to target nucleic acid molecules such as mRNA molecules employs techniques well known in the art. However, in one embodiment of this invention, nucleic acids are extracted

by precipitation from lysed cells and the mRNA is isolated from the extract using a column which binds the poly-A tails of the mRNA molecules (Maniatis, T. et al., Molecular Cloning; Cold Spring Harbor Laboratory, pp.197-98 (1982)). The mRNA is then exposed to radioactively labelled probe on a nitrocellulose membrane, and the probe hybridizes to and thereby labels complementary mRNA sequences. Binding may be detected by autoradiography or scintillation counting. However, other methods for performing these steps are well known to those skilled in the art, and the discussion above is merely an example.

This invention provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a mammalian glycine transporter so as to prevent translation to the mammalian glycine transporter. The antisense oligonucleotide may have a sequence capable of binding specifically with any sequences of the cDNA molecule whose sequence is shown in Figure 1. This invention further provides an antisense oligonucleotide having a sequence capable of binding specifically with any sequences of an mRNA molecule which encodes a human glycine transporter so as to prevent translation to the human glycine transporter. The antisense oligonucleotide may have a sequence capable of binding specifically with any sequences of the cDNA molecule whose sequence is shown in Figure 7. As used herein, the phrase "binding specifically" means the ability of an antisense oligonucleotide to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. A particular example of an antisense oligonucleotide is an antisense oligonucleotide comprising chemical analogues of nucleotides.

This invention also provides a pharmaceutical composition comprising an effective amount of the oligonucleotide described above effective to reduce expression of a mammalian glycine transporter by passing through a cell membrane and binding specifically with mRNA encoding a mammalian glycine transporter in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. DNA molecules having coding sequences substantially the same as the coding sequence shown in Figure 1 may be used as the oligonucleotides of the pharmaceutical composition. This invention also provides a pharmaceutical composition comprising an effective amount of the oligonucleotide described above effective to reduce expression of a human glycine transporter by passing through a cell membrane and binding specifically with mRNA encoding a human glycine transporter in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane. DNA molecules having coding sequences substantially the same as the coding sequence shown in Figure 7 may be used as the oligonucleotides of the pharmaceutical composition. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The oligonucleotide may be coupled to a substance which inactivates mRNA, such as a ribozyme. The pharmaceutically acceptable hydrophobic carrier capable of passing through cell membranes may also comprise a structure which binds to a transporter specific for a selected cell type and is thereby taken up by cells of the selected cell type. The structure may be part of a protein known to bind a cell-type specific transporter,

for example an insulin molecule, which would target pancreatic cells.

This invention also provides a method of treating abnormalities which are alleviated by reduction of expression of a glycine transporter. This method comprises administering to a subject an effective amount of the pharmaceutical composition described above effective to reduce expression of the glycine transporter by the subject. This invention further provides a method of treating an abnormal condition related to glycine transporter activity which comprises administering to a subject an amount of the pharmaceutical composition described above effective to reduce expression of the glycine transporter by the subject. Several examples of such abnormal conditions are epilepsy, myoclonus, spastic paralysis, muscle spasm, schizophrenia, and cognitive impairment.

Antisense oligonucleotide drugs inhibit translation of mRNA encoding these transporters. Synthetic antisense oligonucleotides, or other antisense chemical structures are designed to bind to mRNA encoding the glycine transporter and inhibit translation of mRNA and are useful as drugs to inhibit expression of glycine transporter genes in patients. This invention provides a means to therapeutically alter levels of expression of mammalian glycine transporters by the use of a synthetic antisense oligonucleotide drug (SAOD) which inhibits translation of mRNA encoding these transporters. Synthetic antisense oligonucleotides, or other antisense chemical structures designed to recognize and selectively bind to mRNA, are constructed to be complementary to portions of the nucleotide sequences shown in Figure 1 of DNA, RNA or of chemically modified, artificial nucleic acids. This

invention further provides a means to therapeutically alter levels of expression of human glycine transporters by the use of a synthetic antisense oligonucleotide drug (SAOD) which inhibits translation of mRNA encoding these transporters. Synthetic antisense oligonucleotides, or other antisense chemical structures designed to recognize and selectively bind to mRNA, are constructed to be complementary to portions of the nucleotide sequences shown in Figure 7 of DNA, RNA or of chemically modified, artificial nucleic acids. The SAOD is designed to be stable in the blood stream for administration to patients by injection, or in laboratory cell culture conditions, for administration to cells removed from the patient. The SAOD is designed to be capable of passing through cell membranes in order to enter the cytoplasm of the cell by virtue of physical and chemical properties of the SAOD which render it capable of passing through cell membranes (e.g., by designing small, hydrophobic SAOD chemical structures) or by virtue of specific transport systems in the cell which recognize and transport the SAOD into the cell. In addition, the SAOD can be designed for administration only to certain selected cell populations by targeting the SAOD to be recognized by specific cellular uptake mechanisms which bind and take up the SAOD only within certain selected cell populations. For example, the SAOD may be designed to bind to a transporter found only in a certain cell type, as discussed above. The SAOD is also designed to recognize and selectively bind to the target mRNA sequence, which may correspond to a sequence contained within the sequence shown in Figure Figure 1 or Figure 7 by virtue of complementary base pairing to the mRNA. Finally, the SAOD is designed to inactivate the target mRNA sequence by any of three mechanisms: 1) by binding to the target mRNA and thus inducing degradation of the mRNA by intrinsic cellular

mechanisms such as RNAse I digestion, 2) by inhibiting translation of the mRNA target by interfering with the binding of translation-regulating factors or of ribosomes, or 3) by inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups, which either degrade or chemically modify the target mRNA. Synthetic antisense oligonucleotide drugs have been shown to be capable of the properties described above when directed against mRNA targets (J.S. Cohen, Trends in Pharm. Sci. 10, 435 (1989); H.M. Weintraub, Sci. Am. January (1990) p. 40). In addition, coupling of ribozymes to antisense oligonucleotides is a promising strategy for inactivating target mRNA (N. Sarver et al., Science 247, 1222 (1990)). An SAOD serves as an effective therapeutic agent if it is designed to be administered to a patient by injection, or if the patient's target cells are removed, treated with the SAOD in the laboratory, and replaced in the patient. In this manner, an SAOD serves as a therapy to reduce transporter expression in particular target cells of a patient, in any clinical condition which may benefit from reduced expression of glycine transporters.

This invention provides an antibody directed to the mammalian glycine transporter. This antibody may comprise, for example, a monoclonal antibody directed to an epitope of a mammalian glycine transporter present on the surface of a cell, the epitope having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the mammalian glycine transporter included in the amino acid sequence shown in Figure 1. This invention further provides an antibody directed to the human glycine transporter. This antibody may comprise, for example, a monoclonal antibody directed to an epitope of a human glycine transporter present on

the surface of a cell, the epitope having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the human glycine transporter included in the amino acid sequence shown in Figure 7. Amino acid sequences may be analyzed by methods well known to those skilled in the art to determine whether they produce hydrophobic or hydrophilic regions in the proteins which they build. In the case of cell membrane proteins, hydrophobic regions are well known to form the part of the protein that is inserted into the lipid bilayer which forms the cell membrane, while hydrophilic regions are located on the cell surface, in an aqueous environment. Therefore antibodies to the hydrophilic amino acid sequences shown in Figure 1 will bind to a surface epitope of a mammalian glycine transporter as described. Antibodies to the hydrophilic amino acid sequences shown in Figure 7 will bind to a surface epitope of a human glycine transporter as described. Antibodies directed to the mammalian or human glycine transporters may be serum-derived or monoclonal and are prepared using methods well known in the art. For example, monoclonal antibodies are prepared using hybridoma technology by fusing antibody producing B cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired antibody. Cells such as NIH3T3 cells or Ltk^- cells may be used as immunogens to raise such an antibody. Alternatively, synthetic peptides may be prepared using commercially available machines and the amino acid sequences shown in Figure 1 and Figure 7. As a still further alternative, DNA, such as a cDNA or a fragment thereof, may be cloned and expressed and the resulting polypeptide recovered and used as an immunogen. These antibodies are useful to detect the presence of mammalian glycine transporters encoded by the isolated DNA, or to

inhibit the function of the transporters in living animals, in humans, or in biological tissues or fluids isolated from animals or humans.

This invention also provides a pharmaceutical composition which comprises an effective amount of an antibody directed to an epitope of the mammalian glycine transporter, effective to block binding of naturally occurring substrates to the glycine transporter, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to an epitope of a mammalian glycine transporter present on the surface of a cell which has an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the mammalian glycine transporter included in the amino acid sequence shown in Figure 1 is useful for this purpose.

This invention further provides a pharmaceutical composition which comprises an effective amount of an antibody directed to an epitope of a glycine transporter, effective to block binding of naturally occurring substrates to the glycine transporter, and a pharmaceutically acceptable carrier. A monoclonal antibody directed to an epitope of a mammalian glycine transporter present on the surface of a cell which has an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of the mammalian glycine transporter included in the amino acid sequence shown in Figure 1 is useful for this purpose.

This invention also provides a method of treating abnormalities in a subject which are alleviated by reduction of expression of a mammalian glycine transporter which comprises administering to the subject an effective amount of the pharmaceutical composition described above

effective to block binding of naturally occurring substrates to the glycine transporter and thereby alleviate abnormalities resulting from overexpression of a mammalian glycine transporter. Binding of the antibody to the transporter prevents the transporter from functioning, thereby neutralizing the effects of overexpression. The monoclonal antibodies described above are both useful for this purpose.

This invention further provides a method of treating abnormalities in a subject which are alleviated by reduction of expression of a human glycine transporter which comprises administering to the subject an effective amount of the pharmaceutical composition described above effective to block binding of naturally occurring substrates to the glycine transporter and thereby alleviate abnormalities resulting from overexpression of a human glycine transporter. Binding of the antibody to the transporter prevents the transporter from functioning, thereby neutralizing the effects of overexpression. The monoclonal antibodies described above are both useful for this purpose.

This invention additionally provides a method of treating an abnormal condition related to an excess of glycine transporter activity which comprises administering to a subject an amount of the pharmaceutical composition described above effective to block binding of naturally occurring substrates to the glycine transporter and thereby alleviate the abnormal condition. Some examples of abnormal conditions are epilepsy, myoclonus, spastic paralysis, muscle spasm, schizophrenia, and cognitive impairment.

This invention provides a method of detecting the presence

of a glycine transporter on the surface of a cell which comprises contacting the cell with an antibody directed to the mammalian glycine transporter, under conditions permitting binding of the antibody to the transporter, detecting the presence of the antibody bound to the cell, and thereby the presence of the mammalian glycine transporter on the surface of the cell. This invention further provides a method of detecting the presence of a glycine transporter on the surface of a cell which comprises contacting the cell with an antibody directed to the human glycine transporter, under conditions permitting binding of the antibody to the transporter, detecting the presence of the antibody bound to the cell, and thereby the presence of the human glycine transporter on the surface of the cell. Such a method is useful for determining whether a given cell is defective in expression of glycine transporters on the surface of the cell. Bound antibodies are detected by methods well known in the art, for example by binding fluorescent markers to the antibodies and examining the cell sample under a fluorescence microscope to detect fluorescence on a cell indicative of antibody binding. The monoclonal antibodies described above are useful for this purpose.

This invention provides a transgenic nonhuman mammal expressing DNA encoding a mammalian glycine transporter. This invention also provides a transgenic nonhuman mammal expressing DNA encoding a human glycine transporter. This invention also provides a transgenic nonhuman mammal expressing DNA encoding a mammalian glycine transporter which has a nucleic acid sequence which differs from the sequence of a nucleic acid molecule encoding a glycine transporter at one or more nucleotides and which does not encode a protein having glycine transporter activity. This invention further provides a transgenic nonhuman

mammal expressing DNA encoding a human glycine transporter which has a nucleic acid sequence which differs from the sequence of a nucleic acid molecule encoding a glycine transporter at one or more nucleotides and which does not encode a protein having glycine transporter activity.

This invention also provides a transgenic nonhuman mammal whose genome comprises DNA encoding a mammalian glycine transporter so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a glycine transporter and which hybridizes to mRNA encoding a glycine transporter thereby reducing its translation. This invention further provides a transgenic nonhuman mammal whose genome comprises DNA encoding a human glycine transporter so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding a glycine transporter and which hybridizes to mRNA encoding a glycine transporter thereby reducing its translation. The DNA may additionally comprise an inducible promoter or additionally comprise tissue specific regulatory elements, so that expression can be induced, or restricted to specific cell types. Examples of DNA are DNA or cDNA molecules having a coding sequence substantially the same as the coding sequence shown in Figure 1 and Figure 7. An example of a transgenic animal is a transgenic mouse. Examples of tissue specificity-determining regions are the metallothionein promotor (Low, M.J., Lechan, R.M., Hammer, R.E. et al. Science 231:1002-1004 (1986)) and the L7 promotor (Oberdick, J., Smeyne, R.J., Mann, J.R., Jackson, S. and Morgan, J.I. Science 248:223-226 (1990)).

Animal model systems which elucidate the physiological and behavioral roles of mammalian glycine transporters or human glycine transporters are produced by creating transgenic animals in which the expression of a glycine

transporter is either increased or decreased, or the amino acid sequence of the expressed glycine transporter protein is altered, by a variety of techniques. Examples of these techniques include, but are not limited to: 1) Insertion of normal or mutant versions of DNA encoding a mammalian glycine transporter or the human glycine transporter or homologous animal versions of these genes, by microinjection, retroviral infection or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal (Hogan B. et al. Manipulating the Mouse Embryo, A Laboratory Manual, Cold Spring Harbor Laboratory (1986)) or, 2) Homologous recombination (Capecchi M.R. Science 244:1288-1292 (1989); Zimmer, A. and Gruss, P. Nature 338:150-153 (1989)) of mutant or normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of these glycine transporters. The technique of homologous recombination is well known in the art. It replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native transporter but does express, for example, an inserted mutant transporter, which has replaced the native transporter in the animal's genome by recombination, resulting in underexpression of the transporter. Microinjection adds genes to the genome, but does not remove them, and so is useful for producing an animal which expresses its own and added transporters, resulting in overexpression of the transporter.

One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as M2 medium (Hogan B. et al.

Manipulating the Mouse Embryo, A Laboratory Manual, Cold Spring Harbor Laboratory (1986)). DNA or cDNA encoding a mammalian glycine transporter is purified from a vector (such as plasmid pSVL-rB20a described above) by methods well known in the art. In the case of the human glycine transporter DNA or cDNA is purified from a vector pBluescript-hTC27a by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the trans-gene. Alternatively or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg cell, and is used here only for exemplary purposes.

Since the normal action of transporter-specific drugs is to activate or to inhibit the transporter, the transgenic animal model systems described above are useful for testing the biological activity of drugs directed against these glycine transporters even before such drugs become available. These animal model systems are useful for predicting or evaluating possible therapeutic applications of drugs which activate or inhibit these glycine

transporters by inducing or inhibiting expression of the native or trans-gene and thus increasing or decreasing expression of normal or mutant glycine transporters in the living animal. Thus, a model system is produced in which the biological activity of drugs directed against these glycine transporters are evaluated before such drugs become available. The transgenic animals which over or under produce the glycine transporter indicate by their physiological state whether over or under production of the glycine transporter is therapeutically useful. It is therefore useful to evaluate drug action based on the transgenic model system. One use is based on the fact that it is well known in the art that a drug such as an antidepressant acts by blocking neurotransmitter uptake, and thereby increases the amount of neurotransmitter in the synaptic cleft. The physiological result of this action is to stimulate the production of less transporter by the affected cells, leading eventually to underexpression. Therefore, an animal which underexpresses transporter is useful as a test system to investigate whether the actions of such drugs which result in under expression are in fact therapeutic. Another use is that if overexpression is found to lead to abnormalities, then a drug which down-regulates or acts as an antagonist to the glycine transporter is indicated as worth developing, and if a promising therapeutic application is uncovered by these animal model systems, activation or inhibition of the glycine transporter is achieved therapeutically either by producing agonist or antagonist drugs directed against these glycine transporters or by any method which increases or decreases the expression of these glycine transporters in man.

Further provided by this invention is a method of determining the physiological effects of expressing

varying levels of mammalian glycine transporters which comprises producing a transgenic nonhuman animal whose levels of mammalian glycine transporter expression are varied by use of an inducible promoter which regulates mammalian glycine transporter expression. This invention provides a method of determining the physiological effects of expressing varying levels of human glycine transporters which comprises producing a transgenic nonhuman animal whose levels of human glycine transporter expression are varied by use of an inducible promoter which regulates mammalian glycine transporter expression. This invention also provides a method of determining the physiological effects of expressing varying levels of mammalian glycine transporters which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of mammalian glycine transporter. This invention further provides a method of determining the physiological effects of expressing varying levels of human glycine transporters which comprises producing a panel of transgenic nonhuman animals each expressing a different amount of human glycine transporter. Such animals may be produced by introducing different amounts of DNA encoding a mammalian or human glycine transporter into the oocytes from which the transgenic animals are developed.

This invention also provides a method for identifying a substance capable of alleviating abnormalities resulting from overexpression of a mammalian or human glycine transporter comprising administering the substance to a transgenic nonhuman mammal expressing at least one artificially introduced DNA molecule encoding a mammalian or human glycine transporter and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overexpression of a mammalian or human

glycine transporter. As used herein, the term "substance" means a compound or composition which may be natural, synthetic, or a product derived from screening. Examples of DNA molecules are DNA or cDNA molecules encoding a mammalian transporter encoding a mammalian glycine transporter having a coding sequence substantially the same as the coding sequence shown in Figure 1. Examples of DNA molecules are DNA or cDNA molecules encoding a mammalian transporter encoding a human glycine transporter having a coding sequence substantially the same as the coding sequence shown in Figure 7.

This invention provides a pharmaceutical composition comprising an amount of the substance described supra effective to alleviate the abnormalities resulting from overexpression of mammalian or human glycine transporter and a pharmaceutically acceptable carrier.

This invention further provides a method for treating the abnormalities resulting from overexpression of a mammalian or human glycine transporter which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from overexpression of a mammalian or human glycine transporter.

This invention provides a method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a mammalian or human glycine transporter comprising administering the substance to the transgenic nonhuman mammal described above which expresses only nonfunctional mammalian or human glycine transporter and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of

underexpression of a mammalian or human glycine transporter.

This invention also provides a pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of a mammalian or human glycine transporter and a pharmaceutically acceptable carrier.

This invention further provides a method for treating the abnormalities resulting from underexpression of a mammalian or human glycine transporter which comprises administering to a subject an amount of the pharmaceutical composition described above effective to alleviate the abnormalities resulting from underexpression of a mammalian or human glycine transporter.

This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a specific mammalian glycine transporter allele which comprises: a) obtaining DNA of subjects suffering from the disorder; b) performing a restriction digest of the DNA with a panel of restriction enzymes; c) electrophoretically separating the resulting DNA fragments on a sizing gel; d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a mammalian glycine transporter and labelled with a detectable marker; e) detecting labelled bands which have hybridized to the DNA encoding a mammalian glycine transporter labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f) preparing DNA obtained for diagnosis by steps a-e; and g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA

obtained for diagnosis from step f to determine whether the patterns are the same or different and thereby to diagnose predisposition to the disorder if the patterns are the same. This method may also be used to diagnose a disorder associated with the expression of a specific mammalian glycine transporter allele.

This invention provides a method for diagnosing a predisposition to a disorder associated with the expression of a specific human glycine transporter allele which comprises: a) obtaining DNA of subjects suffering from the disorder; b) performing a restriction digest of the DNA with a panel of restriction enzymes; c) electrophoretically separating the resulting DNA fragments on a sizing gel; d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a human glycine transporter and labelled with a detectable marker; e) detecting labelled bands which have hybridized to the DNA encoding a human glycine transporter labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder; f) preparing DNA obtained for diagnosis by steps a-e; and g) comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and thereby to diagnose predisposition to the disorder if the patterns are the same. This method may also be used to diagnose a disorder associated with the expression of a specific human glycine transporter allele.

This invention provides a method of preparing the isolated glycine transporter which comprises inducing cells to express glycine transporter, recovering the transporter

from the resulting cells, and purifying the transporter so recovered. An example of an isolated glycine transporter is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1 or Figure 7. For example, cells can be induced to express transporters by exposure to substances such as hormones. The cells can then be homogenized and the transporter isolated from the homogenate using an affinity column comprising, for example, glycine or another substance which is known to bind to the transporter. The resulting fractions can then be purified by contacting them with an ion exchange column, and determining which fraction contains transporter activity or binds anti-transporter antibodies.

This invention provides a method of preparing the isolated glycine transporter which comprises inserting nucleic acid encoding glycine transporter in a suitable vector, inserting the resulting vector in a suitable host cell, recovering the transporter produced by the resulting cell, and purifying the transporter so recovered. An example of an isolated glycine transporter is an isolated protein having substantially the same amino acid sequence as the amino acid sequence shown in Figure 1 or Figure 7. This method for preparing glycine transporter uses recombinant DNA technology methods well known in the art. For example, isolated nucleic acid encoding glycine transporter is inserted in a suitable vector, such as an expression vector. A suitable host cell, such as a bacterial cell, or a eukaryotic cell such as a yeast cell, is transfected with the vector. Glycine transporter is isolated from the culture medium by affinity purification or by chromatography or by other methods well known in the art.

This invention provides a method for determining whether a substrate not known to be capable of binding to a mammalian glycine transporter can bind to a mammalian glycine transporter which comprises contacting a mammalian cell comprising a DNA molecule encoding a mammalian glycine transporter with the substrate under conditions permitting binding of substrates known to bind to the glycine transporter, detecting the presence of any of the substrate bound to the glycine transporter, and thereby determining whether the substrate binds to the glycine transporter. The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figure 1 preferably, the mammalian cell is nonneuronal in origin. An example of a nonneuronal mammalian cell is a Cos7 cell. The preferred method for determining whether a substrate is capable of binding to the mammalian glycine transporter comprises contacting a transfected nonneuronal mammalian cell (i.e. a cell that does not naturally express any type of glycine transporter, thus will only express such a transporter if it is transfected into the cell) expressing a glycine transporter on its surface, or contacting a membrane preparation derived from such a transfected cell, with the substrate under conditions which are known to prevail, and thus to be associated with, in vivo binding of the substrates to a glycine transporter, detecting the presence of any of the substrate being tested bound to the glycine transporter on the surface of the cell, and thereby determining whether the substrate binds to the glycine transporter. This response system is obtained by transfection of isolated DNA into a suitable host cell. Such a host system might be isolated from pre-existing cell lines, or can be generated by inserting appropriate components into existing cell lines. Such a transfection system provides a complete response system for

investigation or assay of the functional activity of mammalian glycine transporters with substrates as described above. Transfection systems are useful as living cell cultures for competitive binding assays between known or candidate drugs and substrates which bind to the transporter and which are labeled by radioactive, spectroscopic or other reagents. Membrane preparations containing the transporter isolated from transfected cells are also useful for these competitive binding assays. A transfection system constitutes a "drug discovery system" useful for the identification of natural or synthetic compounds with potential for drug development that can be further modified or used directly as therapeutic compounds to activate or inhibit the natural functions of the mammalian glycine transporter. The transfection system is also useful for determining the affinity and efficacy of known drugs at the mammalian glycine transporter sites.

This invention provides a method for determining whether a compound not known to be capable of specifically binding to a mammalian glycine transporter can specifically bind to the mammalian glycine transporter, which comprises contacting a mammalian cell comprising a plasmid adapted for expression in a mammalian cell which plasmid further comprises a DNA which expresses a mammalian glycine transporter on the cell's surface with the compound under conditions permitting binding of ligands known to bind to a mammalian glycine trasnporter, detecting the presence of any compound bound to the mammalian glycine transporter, the presence of bound compound indicating that the compound is capable of specifically binding to the mammalian glycine transporter.

This invention provides a method for determining whether a compound not known to be capable of specifically binding

to a human glycine transporter can specifically bind to the human glycine transporter, which comprises contacting a mammalian cell comprising a plasmid adapted for expression in a mammalian cell which plasmid further comprises a DNA which expresses a human glycine transporter on the cell's surface with the compound under conditions permitting binding of ligands known to bind to a human glycine trasnporter, detecting the presence of any compound bound to the human glycine transporter, the presence of bound compound indicating that the compound is capable of specifically binding to the human glycine transporter.

This invention also provides a method of screening drugs to identify drugs which specifically interact with, and bind to, a mammalian glycine transporter on the surface of a cell which comprises contacting a mammalian cell comprising a DNA molecule encoding the mammalian glycine transporter on the surface of a cell with a plurality of drugs, detecting those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, the mammalian glycine transporter. The DNA in the cell may have a coding sequence substantially the same as the coding sequence shown in Figure 1. This invention further provides a method of screening drugs to identify drugs which specifically interact with, and bind to, a human glycine transporter on the surface of a cell which comprises contacting a mammalian cell comprising a DNA molecule encoding the human glycine transporter on the surface of a cell with a plurality of drugs, detecting those drugs which bind to the mammalian cell, and thereby identifying drugs which specifically interact with, and bind to, the human glycine transporter. The DNA in the cell may have a coding sequence substantially the same as the coding

sequence shown in Figure 7. Various methods of detection may be employed. The drugs may be "labeled" by association with a detectable marker substance (e.g., radiolabel or a non-isotopic label such as biotin). Preferably, the mammalian cell is nonneuronal in origin. An example of a nonneuronal mammalian cell is a Cos7 cell. Drug candidates are identified by choosing chemical compounds which bind with high affinity to the expressed glycine transporter protein in transfected cells, using radioligand binding methods well known in the art, examples of which are shown in the binding assays described herein. Drug candidates are also screened for selectivity by identifying compounds which bind with high affinity to one particular glycine transporter subtype but do not bind with high affinity to any other glycine transporter subtype or to any other known transporter site. Because selective, high affinity compounds interact primarily with the target glycine transporter site after administration to the patient, the chances of producing a drug with unwanted side effects are minimized by this approach. This invention provides a pharmaceutical composition comprising a drug identified by the method described above and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. Once the candidate drug has been shown to be adequately bio-available following a particular route of administration, for example orally or by injection (adequate therapeutic concentrations must be maintained at the site of action for an adequate period to gain the desired therapeutic benefit), and has been shown to be non-toxic and therapeutically effective in appropriate disease models,

the drug may be administered to patients by that route of administration determined to make the drug bio-available, in an appropriate solid or solution formulation, to gain the desired therapeutic benefit.

Applicants have identified individual transporter subtype proteins and have described methods for the identification of pharmacological compounds for therapeutic treatments. Pharmacological compounds which are directed against specific transporter subtypes provide effective new therapies with minimal side effects.

Elucidation of the molecular structure of the neural glycine transporter is an important step in the understanding of glycinergic neurotransmission. This disclosure reports the isolation, amino acid sequence, and functional expression of a cDNA clone from rat brain which encodes a glycine transporter. This disclosure further reports the isolation, amino acid sequence, and functional expression of a cDNA clone from human brain which encodes a glycine transporter. The identification of these transporters will play a pivotal role in elucidating the molecular mechanisms underlying glycinergic transmission and neural modulation and should also aid in the development of novel therapeutic agents.

A complementary DNA clone (designated rB20a) encoding a transporter for glycine has been isolated from rat brain, and its functional properties have been examined in mammalian cells. The nucleotide sequence of rB20a predicts a protein of 638 amino acids, with 12 highly hydrophobic regions compatible with membrane-spanning domains. When incubated with 50 nM [^{3}H]glycine, COS cells transiently transfected with rB20a accumulate 50-fold as much radioactivity as non-transfected control cells. The

transporter encoded by rB20a displays high-affinity for glycine (K_m≈100uM) and is dependent on external sodium and chloride. In addition complementary DNA clone (designated hTC27a) encoding a transporter for glycine has been isolated from human brain. Analysis of the glycine transporter structure and function provides a model for the development of drugs useful as cognitive enhancers, and for the treatment of epilepsy and other neurological disorders.

This invention identifies for the first time a new transporter protein, its amino acid sequence, and its mammalian gene and its human gene. The information and experimental tools provided by this discovery are useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for this new transporter protein, its associated mRNA molecule or its associated genomic DNA. The information and experimental tools provided by this discovery will be useful to generate new therapeutic agents, and new therapeutic or diagnostic assays for this new transporter protein, its associated mRNA molecule, or its associated genomic DNA.

Specifically, this invention relates to the first isolation of a mammalian cDNA and genomic clone encoding a glycine transporter. A new mammalian gene for the transporter identified herein as rB20a has been identified and characterized, and a series of related cDNA and genomic clones have been isolated. In addition, the mammalian glycine transporter has been expressed in Cos7 cells by transfecting the cells with the plasmid pSVL-rB20a. The pharmacological properties of the protein encoded have been determined, and these properties classify this protein as a glycine transporter. Mammalian cell lines expressing this mammalian glycine transporter

at the cell surface have been constructed, thus establishing the first well-defined, cultured cell lines with which to study this glycine transporter.

This invention further relates to the first isolation of a human cDNA and genomic clone encoding a glycine transporter. The new human gene for the human transporter identified herein as hTC27a has been identified and characterized.

The invention will be better understood by reference to the Experimental Details which follow, but those skilled in the art will readily appreciate that the specific experiments detailed are only illustrative, and are not meant to limit the invention as described herein, which is defined by the claims which follow thereafter.

Experimental Details

Materials and Methods

Cloning and Sequencing of Rat Glycine Transporter: A rat brain cDNA library in the Lambda ZAP II vector (Stratagene, La Jolla, CA) was screened at low stringency using overlapping probes representing the coding region of the rat GABA transporter cDNA (Guastella et al., 1990). Exact primers were used to generate PCR products encoding the GABA transporter from randomly-primed rat brain cDNA. Three sets of primers were designed from nucleotide sequence of the rat GABA transporter cDNA (Guastella et al., 1990) such that three products represented the entire coding region. Primer set one was made as a sense oligonucleotide derived from nucleotides -125 to -109 and an antisense oligonucleotide derived from nucleotides 721-737 to generate a PCR product of 862bp; primer set two was composed of sense and antisense oligonucleotides derived from nucleotides 613-629 and 1417-1433, respectively, to generate a PCR product of 821bp; primer set three was composed of sense and antisense oligonucleotides derived from nucleotides 1318-1334 and 1860-1876, respectively, to generate a PCR product of 559bp. The 559bp PCR product was gel purified, subcloned, and sequenced to confirm its identity; the others were gel purified and used directly as probes. All three probes were labeled with ^{32}P by the method of random priming (Feinberg and Vogelstein, 1983). Hybridization was performed at 40°C in a solution containing 25% formamide, 10% dextran sulfate, 5X SSC (1X SSC is 0.15 M sodium chloride, 0.015 M sodium citrate), 1X Denhardt's (0.02% polyvinylpyrrolidone, 0.02% Ficoll, and 0.02% bovine serum albumin), and 100 μg/ml of sonicated salmon sperm DNA. The filters were washed at 40°C in 0.1X SSC containing 0.1% sodium dodecyl sulfate (SDS) and

exposed at -70°C to Kodak XAR film in the presence of one intensifying screen. Lambda phage hybridizing to the probe were plaque purified and screened with the same probe mixture at high stringency to eliminate exact matches. Candidate clones were converted to phagemids by in vivo excision with f1 helper phage. Nucleotide sequences of double-stranded cDNAs in pBluescript were analyzed by the Sanger dideoxy nucleotide chain-termination method (Sanger, 1977) using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio).

Expression: Two cDNA clones which collectively span the entire coding region of the glycine transporter gene, including 63 base pairs of 5' untranslated sequence and 189 base pairs of 3' untranslated sequence, were identified. These two clones were constructed into a full-length clone (designated rB20a) by ligation at their internal Nco I sites and then cloned into the eukaryotic expression vector pSVL (Pharmacia LKB Biotechnology, Piscataway, NJ). Transient transfection of COS cells was carried out using DEAE-dextran with DMSO according to the method of Lopata et al. (1984) with minor modifications. COS cells were grown in six-well plates (37°C.,5%CO_2) in high glucose Dulbecco's modified Eagle medium supplemented with 10% bovine calf serum, 100 U/ml penicillin G, and 100 µg/ml streptomycin sulfate. Cells were routinely used two days after transfection for transport studies.

Transport Studies: To measure glycine transport, COS cells grown in 6-well plates (well diameter = 35mm) were washed 3X with HEPES-buffered saline (HBS, in mM: NaCl, 150; HEPES, 20; $CaCl_2$, 1; glucose, 10; KCl, 5; $MgCl_2$, 1; pH 7.4) and allowed to equilibrate in a 37°C water bath. After 10 minutes the medium was removed and a solution containing [^{3}H]glycine (New England Nuclear, sp. activity

= 45Ci/mmole) and required drugs in HBS was added (1.5 ml/well). Plates were incubated at 37°C for 10 or 20 minutes, then washed rapidly 3x with HBS. Cells were solubilized with 0.05% sodium deoxycholate/0.1N NaOH (1 ml/well), 0.5ml aliquots were removed, neutralized with 1N HCl, and radioactivity was determined by scintillation counting. Protein was quantified in an aliquot of the solubilized cells with the Bradford Reagent (Biorad, Richmond, CA), according to the manufacturer's directions. Non-specific uptake was defined in parallel wells with 1mM unlabeled glycine, and was subtracted from total uptake (no competitor) to yield specific uptake; all data represent specific uptake.

Northern Blot Analysis: Total cellular RNA was isolated from rat tissues using RNAzol (Cinna/Biotecx Laboratories Inc.; Houston, TX) as outlined by the manufacturer. Denatured RNA samples (~30μg) were separated in a 1.2% agarose gel containing 3.3% formaldehyde. RNAs were transferred to nitrocellulose membranes (Schleicher and Schuell, Keene, NH) by overnight capillary blotting in 10X SSC. Northern blots were rinsed and then baked for 2 hours at 80°C under vacuum. Prehybridization was for 1 hour at 65°C in a solution containing 50% formamide, 2X SSC, 1X Denhardt's, 0.1% SDS, 20mM sodium phosphate, and 10mM EDTA. Blots were hybridized overnight at 42°C with ^{32}P-labeled DNA probes (randomly primed) in prehybridization mixture containing 125 μg/ml sonicated salmon sperm DNA. The blots were washed successively in 2X SSC/1% SDS and 0.1X SSC/1% SDS at 65°C, then exposed to Kodak XAR-5 film with one intensifying screen at -90°C for up to one week.

In Situ Hybridization: Male Sprague-Dawley rats (Charles River) were decapitated and the brains rapidly frozen in

isopentane. Sections were cut on a cryostat, thaw-mounted onto poly-L-lysine coated coverslips, and stored at -80°C until use. Tissue was fixed in 4% paraformaldehyde, treated with 5mM dithiothreitol (DTT), acetylated (0.25% acetic anhydride in 0.1M triethanolamine), and dehydrated. Tissue was prehybridized (1 hour, 40°C) in a solution containing 50% formamide, 4X SSC (0.6M NaCl/0.06M sodium citrate), 1X Denhardt's solution (0.2% polyvinylpyrrolidine, 0.2% Ficoll, 0.2% bovine serum albumin), 50mM DTT, 500µg/ml salmon sperm DNA, 500µg/ml yeast tRNA, 10% dextran sulfate, then hybridized overnight with ^{35}S-labeled anti-sense oligonucleotides (45mers) in the same solution. After washing and dehydration, sections were apposed to Kodak X-OMAT AR film for 4 days at -20°C. To verify the specificity of the hybridization signal, parallel tissues were pretreated with 100 µg/ml RNase A (37°, 30 minutes) prior to hybridization. Two different oligonucleotides designed to separate regions of the glycine transporter (loop region between transmembrane domains III and IV, 3'untranslated region) showed identical patterns of hybridization.

Use of PCR to Identify Human cDNA Libraries for Screening: For hGlycine, the sequences of the rat PCR primers were 5'- (ATGGCTGTGGCTCACGGACCTGTGG) and 5'- (TGAAGACTTGACTCCTCGAATGAGGCAGAG). PCR reactions were carried out in a buffer containing 20mM Tris (pH 8.3), 50 mM KCl, 1.5mM $MgCl_2$, 0.001% gelatin, 2mM dNTP's, 1µM each primer, Taq polymerase, and an aliquot of a lambda phage library, water, or a control plasmid for 40 cycles of 94°C. for 2 min., 50°C. for 2 min., and 72°C. for 3 min. PCR reactions were carried out as described above for 40 cycles of 94°C. for 2 min., 40°C. for 2 min., and 72°C. for 3 min. PCR products were separated by electrophoresis in 1.2% agarose gels, blotted to nylon membranes (Zeta-

Probe GT; Bio-Rad Laboratories, Richmond, CA), and hybridized at 40°C. overnight with ^{32}P-labeled oligonucleotide probes (overlapping 45mers) in a solution containing 25% formamide, 10% dextran sulfate, 5X SSC, 1X Denhardt's, and 100 μg/ml of sonicated salmon sperm DNA. The sequences of the oligonucleotides corresponded to amino acids 204-226 of the rat glycine transporter. Blots were washed at low stringency (0.1X SSC, 40°C.) and exposed to Kodak XAR film for up to three days with one intensifying screen at -70°C.

Isolation and Sequencing of Human Clones: Human cDNA libraries in the Lambda ZAP or Lambda ZAP II vector (Stratagene, La Jolla, CA) that were identified as containing hGlycine were screened under reduced stringency (25% formamide, 40°C. hybridization; 0.1X SSC, 40°C. wash). Hybridizing lambda phage were plaque purified and converted to phagemids by in vivo excision with f1 helper phage. Nucleotide sequences of double-stranded cDNAs in pBluescript were analyzed by the Sanger dideoxy nucleotide chain-termination method (Sanger, 1977) using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio).

Results

To clone the glycine transporter, a rat brain cDNA library was screened at low stringency with probes encoding the rat GABA transporter (Guastella et al., 1990). Of 48 clones isolated, ten were identified which hybridized at low but not at high stringency with the GABA transporter probes. DNA sequence analysis revealed that seven of these clones contained overlapping fragments. Two of the clones were identified which together comprised a 2.2 kb sequence (rB20a) with an open reading frame of 1917 base pairs. Comparison of this sequence with the rat GABA transporter revealed 55-60% nucleotide identity within the coding region. Searches of Genbank and EMBL data bases demonstrated that the nucleotide sequence was novel and that the two most closely related sequences were the rat GABA transporter (Guastella et al., 1990) and the human norepinephrine transporter (Pacholczyk et al., 1991).

The nucleotide and deduced amino acid sequence and proposed membrane topology of the protein encoded by rB20a is shown in Figure 1. An open reading frame extending from an ATG start codon at position 1 to a stop codon at position 1917 can encode a protein 638 amino acids in length, having a relative molecular mass (M_r) of approximately 72,000. Hydropathy analysis indicates the presence of 12 hydrophobic domains which may represent membrane spanning segments (data not shown). We have modeled the glycine transporter with both termini inside the cell, similar to the membrane topology proposed for the GABA (Guastella et al., 1990) and noradrenaline (Pacholczyk et al., 1991) transporters. Of six potential sites for Asn-linked glycosylation, four are found in the loop between the third and fourth transmembrane domains which is predicted to be extracellular. Alignment with

the GABA transporter revealed 45% amino acid identity (68% homology with conservative substitutions). Comparison of rB20a with the human norepinephrine transporter (Pacholczyk et al., 1991) showed a similar degree of amino acid identity (42%) (Figure 2). These data suggested that the new sequence encodes a novel transporter expressed in the brain. To explore this possibility, the sequence was placed in a mammalian expression vector (pSVL), transfected into COS cells, and screened for transport of a variety of radiolabeled neurotransmitters and amino acids.

COS cells transiently transfected with rB20a (COS/rB20a) accumulated more [^{3}H]glycine than non-transfected control cells (Figure 3). During a 20 minute incubation (37°C) with a low concentration of [^{3}H]glycine (50-100nM), specific uptake was increased 54±6-fold over control (mean±SEM,n=6 experiments); a representative experiment is shown in Figure 3. Specific uptake represented 45±4 and 87±1% (mean±SEM, n=6) of total uptake in control and transfected cells, respectively, and the absolute levels of non-specific uptake were similar in both cases. The high percentage of specific uptake observed in transfected cells demonstrates that the enhanced uptake resulting from expression of rB20a displays saturability. Uptake of [^{3}H]glycine was not increased following transfection with either a plasmid lacking the insert or containing an irrelevant insert (not shown), indicating that the enhanced uptake was specific for rB20a and was not due to non-specific perturbation of the membrane. Further, expression of rB20a did not significantly alter the uptake of [^{3}H]GABA, [^{3}H]histamine, [^{3}H]glutamate, [^{3}H]tyrosine, [^{3}H]norepinephrine, [^{3}H]5-HT, or [^{3}H]dopamine (data not shown). The transport of [^{3}H]glycine was decreased ≥95% when Na^+ was replaced by Li^+ (Figure 3) or choline (not

shown), or when Cl^- was replaced by acetate and gluconate (Figure 3). Thus, the glycine transporter encoded by rB20a displays an absolute requirement for Na^+ and Cl^-, similar to the cloned GABA transporter (Guastella et al., 1990). Taken together, these data indicate that rB20a encodes a saturable, sodium- and chloride-dependent glycine transporter.

The kinetics of uptake of 50nM [^{3}H]glycine in rB20a/COS cells are shown in Figure 4A. The specific accumulation of [^{3}H]glycine was linear for the first few minutes and approached saturation by about 5 minutes. To determine the affinity of glycine for the cloned transporter, COS cells transfected with rB20a were incubated with various concentrations of [^{3}H]glycine and the specific accumulation of radioactivity was determined. A representative experiment is shown in Figure 4B in which it can be seen that uptake saturated at higher concentrations of glycine, as expected for a carrier-mediated process. Non-linear regression analysis of the data indicate a K_M of 123 μM and a V_{MAX} of 28 nmoles per minute per mg protein (mean of 2 experiments).

To determine the pharmacological specificity of the transporter encoded by rB20a, we examined the ability of various agents to compete for the uptake of [^{3}H]glycine by COS cells transfected with rB20a (Table 1).

TABLE 1 *Pharmacological Specificity of [^{3}H]glycine Uptake In COS-7 Cells Transfected with rB20a*

Inhibitor[a]	concentration	%displacement
L-alanine	1mM	2
dopamine	1mM	0
GABA	1mM	0
glycine	1mM	100
L-glutamate	1mM	0
glycine ethyl ester	10μM	0
	100μM	0
	1mM	32
glycine methyl ester	10μM	0
	100μM	0
	1mM	42
histamine	1mM	0
α-(methylamino) isobutyric acid	1mM	3
(-)-norepinephrine	1mM	0
sarcosine	10μM	23
	100μM	64
	1mM	100
L-serine	1mM	4

[a] COS-7 cells transfected with rB20a encoding the glycine transporter were incubated for 10 minutes (37°C) with 50nM [^{3}H]glycine and the indicated compounds. Non-specific uptake was determined with 1mM glycine. Data show percent displacement of specific [^{3}H]glycine uptake.

Glycine is a substrate for multiple amino acid transport systems in various tissues, therefore it was important to determine the relationship of the cloned transporter to previously identified systems. Neither α-(methylamino)isobutyric acid (1mM), a substrate for system A, nor L-serine (1mM), a substrate for system ASC, significantly competed for [^{3}H]glycine uptake. Sarcosine (N-methylglycine) inhibited specific [^{3}H]glycine transport 23%, 64% and 100% at 10μM, 100μM, and 1mM, respectively, consistent with an IC_{50} of approximately 50μM. The ethyl- and methyl-esters of glycine were less potent than glycine, inhibiting specific transport 32% and 42% at 1mM, respectively; no inhibition was seen at 10μM and 100μM. Other agents tested did not compete for [^{3}H]glycine uptake. These data indicate that rB20a encodes a glycine-specific transporter.

To define the distribution of the mRNA encoding the glycine transporter we carried out Northern blot analysis of total RNA isolated from a variety of rat brain regions and peripheral tissues (Figure 5). A single transcript (≈ 3.8 kb) which hybridized at high stringency with the glycine transporter cDNA was present in all CNS samples, including total brain, midbrain, hind brain, cerebellum, and spinal cord, with lower levels in forebrain. Following normalization of RNA levels by reprobing with a cDNA encoding cyclophilin (Danielson et al., 1988), the adjusted levels of glycine mRNA in the spinal cord and cerebellum were determined to be roughly equivalent to those found in hindbrain and midbrain. The transcript was not detectable in spleen, kidney, or aorta. A very light signal was detected in liver; this reflects either cross-hybridization with a related gene, or extremely low expression of the glycine transporter mRNA. These data

suggest that the glycine transporter mRNA is expressed primarily in the nervous system.

To more precisely determine the localization of the glycine transporter, in situ hybridization of specific antisense probes was examined in coronal sections of the rat CNS (Figure 6). Glycine transporter mRNA was observed at all brain levels, though the distribution displayed considerable regional heterogeneity. Moderate to high levels of mRNA were detected in spinal cord, brain stem, and midbrain, areas in which the role of glycine in inhibitory neurotransmission is well established. The globus pallidus and hypothalamus were moderately labeled, whereas light labeling was observed in the thalamus and striatum; the substantia nigra was devoid of label. The neocortex displayed light, diffuse labeling at all rostro-caudal levels. Dense labeling was observed in the mitral cell layer of the olfactory bulb and the granular layer of the cerebellum. Surprisingly, heavy labeling was observed in the pyramidal cell layer of the hippocampal formation (dentate gyrus, CA1, CA2, and CA3) (Figure 6), an area in which classical glycine receptors are absent or in low abundance (Malosio et al., 1991; van den Pol and Gorcs, 1988). Rather, the labeling pattern in the hippocampus corresponds to that of the glycine modulatory site of the NMDA receptor (Monoghan, 1990).

To obtain a cDNA clone encoding the human glycine transporter (hGlycine) we used PCR primers based on the nucleotide sequence of the rat glycine transporter cDNA to detect the presence of hGlycine in human cDNA libraries. PCR was carried out at a reduced annealing temperature to allow mismatches between rat and human sequences (see Experimental Procedures); amplified hGlycine sequences were detected by hybridization at low stringency with

radiolabeled oligonucleotides representing the rat glycine transporter sequence. A human temporal cortex cDNA library (Stratagene) was identified and screened at low stringency with the same probes, resulting in isolation of a partial cDNA clone (hTC27a) containing the major portion of the coding region of hGlycine. The hGlycine nucleotide sequence from this clone and the deduced amino acid sequence based on translation of a long open reading frame is shown in Figure 7. The sequence includes 936 base pairs of coding region (312 amino acids) and 45 base pairs of 5' untranslated region. Comparison with the rat glycine transporter amino acid sequence reveals 95% identity over the region encoded by the clone, which includes the initiating methionine (N-terminus) and predicted transmembrane domains 1-5 of the human glycine transporter. Compared with the rat, the N-terminus of the human glycine transporter is predicted to contain 14 additional amino acids based on a different predicted site for translation initiation in the human sequence.

Discussion

Despite their importance in synaptic transmission, our understanding of the molecular nature of neurotransmitter transporters has lagged behind that of neurotransmitter receptors. Our identification of a cDNA clone encoding a glycine transporter, together with the recent cloning of transporters for GABA (Guastella et al., 1990), norepinephrine (Pacholczyk et al., 1991), dopamine (Kilty et al., 1991; Shimada et al., 1991), and serotonin (Blakely et al., 1991; Hoffman et al., 1991), provides a framework for defining the structural features of this class of membrane proteins.

The glycine transporter cloned from rat brain displays striking sequence similarity to the other members of the transporter family. Alignment of the amino acid sequence of the glycine transporter with those of the GABA and norepinephrine transporters (Figure 2) reveals multiple domains which are highly conserved within the family. Despite differing substrate specificities, over half of the residues shared between the GABA and norepinephrine carriers are also present in the glycine transporter, and the majority of these are common to all five cloned transporters. It seems unlikely that such regions are directly involved in substrate recognition, but rather may subserve a common transport function. A characteristic which distinguishes the neurotransmitter transporters from other similarly modeled nutrient transporters, such as the facilitated glucose carriers (Kayano et al., 1990), is the large extracellular loop between transmembrane domains 3 and 4, which has several potential glycosylation sites. Amino acid sequences in this loop and in transmembrane domains 9-11 are more divergent than in many other regions, raising the possibility that these domains

contribute to specificity of substrate recognition.

In addition to its signalling roles, glycine also functions as an amino acid constituent of proteins in both neural and non-neural tissues. Northern blot analysis suggests that the cloned glycine transporter is neural-specific and thus is distinct from "system gly", a glycine-specific transport system present in various non-neural tissues such as hepatocytes (Christensen and Handlogten, 1981; Moseley et al., 1988) and red blood cells (Felipe et al., 1990). The pharmacological specificity of the cloned glycine transporter (Table 1) is similar to that observed for the high-affinity glycine transporter present in cultured glial cells (Zafra and Gimenez, 1989) and to the reconstituted transporter isolated from spinal cord (Lopez-Corcuera and Aragon, 1989), and clearly distinguishes it from two of the classical amino acid transporter systems, system A and system ASC (Christensen, 1984), both of which can transport glycine as well as other amino acids. Additionally, the affinity of the cloned transporter for glycine (Km =123uM) is nearly identical to that of the high-affinity transporter present in glial cell cultures (95uM; Zafra and Gimenez, 1989) and differs by only 2-fold from the high-affinity transporter in rat brain synaptosomes (50uM; Mayor et al., 1981). Taken together, these data support a role for the cloned glycine transporter in neurotransmission, consistent with its high degree of structural similarity to other neurotransmitter transporters. The identification of a neural-specific high-affinity glycine transporter suggests that it may be possible to design selective, centrally acting glycine uptake inhibitors.

Localization studies of the mRNA for the glycine

transporter reveal that it is not only present in spinal cord and brain stem, where it presumably participates in classical inhibition, but it is also extensively expressed in hippocampus and cortex, areas in which classical glycine inhibitory receptors are thought to be absent or in low abundance (Malosio et al., 1991; van den Pol and Gorcs, 1988). Rather, these areas contain high levels of NMDA receptor-associated glycine binding sites (Monoghan, 1990; Moriyoshi et al., 1991; Kumar et al., 1991) suggesting that the glycine transporter modulates NMDA receptors and could serve to regulate cognitive processes such as memory storage. Our finding of high levels of glycine transporter mRNA in the hippocampal formation suggests that the endogenous level of glycine in the extracellular space may be modulated by the transporter. The ability to modulate glycine levels and thereby to modulate the functional effectiveness of the NMDA receptor may have importance for regulating higher nervous system processes.

Recently, a glycine transporter cDNA that is similar but not identical to that cloned by Smith et al. (1992) was cloned from both rat (Guastella et al., 1992) and mouse (Liu et al., 1992a). These isoforms may result from alternative splicing and could provide a means for regulating tissue-specific expression. In addition to those for glycine, several additional transporters have been cloned which exhibit significant sequence homology with previously cloned neurotransmitter transporters. cDNA and genomic clones representing the mouse homologues of the GABA transporter GAT-1 were recently reported (Liu et al., 1992). We recently reported the cloning and expression of two novel high-affinity GABA transporters from rat brain, designated GAT-2 and GAT-3 (Borden et al., 1992). A β-alanine-sensitive GABA transporter from rat

brain has been cloned by Clark et al., (1992) that exhibits 100% amino acid identity with the rat GAT-3 sequence reported by Borden et al. (1992). A high-affinity L-proline transporter was reported by Fremeau et al. (1992), supporting a role for L-proline in excitatory neurotransmission. A rat cDNA identified as a choline transporter was reported by Mayser et al. (1992). A taurine transporter cDNA was recently cloned from dog kidney cells (Uchida et al., 1992) which is 90% identical to the rat taurine transporter amino acid sequence reported by Smith et al. (1992a). Finally, a cDNA encoding a mouse GABA transporter was recently cloned by Lopez-Corcuera et al. (1992); the transporter encoded by this cDNA is 88% identical to the dog betaine transporter (Yamauchi et al., 1992).

The use of human gene products in the process of drug development offers significant advantages over those of other species, which may not exhibit the same pharmacologic profiles. To facilitate this human target-based approach to drug design in the area of inhibitory amino acid transporters, we used the nucleotide sequence of the rat brain high-affinity glycine transporter (Smith et al., 1992) to clone the human glycine transporter. The cloning and expression of the human brain glycine transporter will allow comparison of its pharmacological profile with that of the rat glycine transporter, and also provide a means for understanding and predicting the mechanism of action of glycine uptake inhibitors as human therapeutics.

REFERENCES

Aprison, M.H. (1990). The discovery of the neurotransmitter role of glycine; in Glycine Transmission

(Ottersen, O.P. and Storm-Mathisen, J., ed), John Wiley and Sons, New York, pp. 1-24.

Becker, C.-M. (1990). Disorders of the inhibitory glycine receptor: the *spastic* mouse. FASEB. J. 4, 2767-2771.

Bennett, J.P, Jr., Mulder, A.H., and Snyder, S.H. (1974). Neurochemical correlates of synaptically active amino acids. Life Sci. 15, 1045-1056.

Blakely, R. D., Berson, H. E., Fremeau, Jr., R. T., Caron, M. G., Peek, M. M., Prince, H. K., and Bradley, C. C. (1991). Cloning and expression of a functional serotonin transporter from rat brain. Nature 354, 66-70.

Borden, L.A., K.E. Smith, P.R. Hartig, T.A. Branchek, and R.L. Weinshank (1992) *J. Biol. Chem.* 267, 21098-21104.

Christensen, H.N. (1984). Organic ion transport during seven decades. The amino acids. Biochim. Biophys. Acta 779, 255-269.

Christensen, H.N. and Handlogten, M.E. (1981). Role of system gly in glycine transport in monolayer cultures of liver cells. Biochem. Biophys. Res. Comm. 98, 102-107.

Clark, J.A., A.Y. Deutch, P.Z. Gallipoli, and S.G. Amara (1992) *Neuron* 9, 337-348.

Cohen, J.S. (1989) Trends in Pharm. Sci. 10, 435.

Collingridge, G.L. and Bliss, T.V.P. (1987). NMDA receptors-their role in long-term potentiation. Trends Neurosci. 10, 288-293.

Cortes, R. and Palacios, J.M. (1990). Autoradiographic mapping of glycine receptors by [^{3}H]strychnine binding; in Glycine Transmission (Ottersen, O.P. and Storm-Mathisen, J., ed), John Wiley and Sons, New York, pp.240-263.

Daly, E.C. (1990). The biochemistry of glycinergic neurons; in Glycine Transmission (Ottersen, O.P. and Storm-Mathisen, J., ed), John Wiley and Sons, New York, pp. 25-66.

Danielson, P.E., Forss-Petter, S., Brow, M.A., Calavetta, L., Douglass, J., Milner, R.J. and Sutcliffe, J.G. (1988). p1B15: A cDNA clone of the rat mRNA encoding cyclophilin. DNA 7, 261-267.

Debler, E.A.and Lajtha, A. (1987) J. Neurochem. 48, 1851-1856.

Feinberg, A. P., and Vogelstein, B. (1988). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132, 6-13.

Felipe, A., Vinas,O., and Remear, X. (1990). Changes in glycine and leucine transport during red cell maturation in the rat. Neurosci. Reports 10, 209-216.

Fletcher, E.J. Beart, P.M., and Lodge, D. (1990). Involvement of glycine in excitatory neurotransmission; in Glycine Transmission (Ottersen, O.P. and Storm-Mathisen, J., ed), John Wiley and Sons, New York, pp. 193-219.

Fremeau, R.T.,Jr., M.G. Caron, and R.D. Blakely (1992) *Neuron* 8, 915-926.

Grenningloh, G., Rienitz, A., Schmitt, B., Methfessel, C.,

Zensen, M., Beyreuther, K., Gundelfinger, E.D., and Betz, H. (1987). The strychnine-binding subunit of the glycine receptor shows homology with nicotinic acetylcholine receptors. Nature 328, 215-220.

Guastella, J., N. Brecha, C. Wiegmann, H.A. Lester, and N. Davidson (1992) *Proc. Natl. Acad. Sci. USA* 89, 7189-7193.

Guastella, J., Nelson, N., Nelson, H., Czyzyk, L., Keynan, S., Miedel, M.C., Davidson, N., Lester, H.A., and Kanner, B.I. (1990). Cloning and expression of a rat brain GABA transporter. Science 249, 1303-6.

Gundlach, A.L. (1990). Disorder of the inhibitory glycine receptor: inherited myoclonus in Poll Hereford calves. FASEB J. 4, 2761-2766.

Handelmann, G. E., Nevins, M.E., Mueller, L.L., Arnolde, S.M. and Cordi, A.A. (1989). Milacemide, a glycine prodrug, enhances performance of learning tasks in normal and amnestic rodents. Pharmacol., Biochem. and Behavior 34, 823-828.

Hardy, J.A., Barton, A., Lofdahl, E., Cheetham, S.C., Johnston, G.A.R., and Dodd, P.R. (1986) J. Neurochem. 47, 460-467.

Hoffman, B. J., Mezey, E., and Brownstein, M. J. (1991). Cloning of a serotonin transporter affected by antidepressants. Science 254, 579-580.

Horn, A.S. (1990). Dopamine uptake: a review of progress in the past decade. Prog. Neurobiol. 34, 387-400.

International Patent Application Number WO 90/06047.

Johnson, J.W. and Ascher, P. (1987). Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325, 529-531.

Kanner, B.I, and Shuldiner, S. (1987). Mechanism of transport and storage of neurotransmitters. CRC crit. Rev. Biochem. 22, 1-38.

Kayano, T., Burant, C. F., Fukumoto, H., Gould, G. W., Fan, Y.-S., Eddy, R. L., Byers, M. G., Shows, T. B., Seins, S., and Bell, G. I. (1990). Human facilitative glucose transporters: Isolation, functional characterization, and gene localization of cDNAs encoding an isoform (GLUT5) expressed in small intestine, kidney, muscle, and adipose tissue and an unusual glucose transporter pseudogene-like sequence (GLUT6). J. Biol Chem. 265, 13276-13282.

Kilberg, M.S. (1982) J. Membrane. Biol. 69, 1-12.

Kilty, J. E., Lorang D., and Amara, S. G. (1991). Cloning and expression of a cocaine-sensitive rat dopamine transporter. Science 254, 578-579.

Kumar, K.N., Tilakaratne, N., Johnson. P.S., Allen, A.E., and Michaelis, E.K. (1991). Cloning of the cDNA for the glutamate-binding subunit of an NMDA receptor complex. Nature 354, 70-73.

Liu, Q.-R., H. Nelson, S. Mandiyan, B. Lopez-Corcuera, and N. Nelson (1992a) *FEBS Letters* 305, 110-114.

Liu, Q.-R., S. Mandiyan, H. Nelson, and N. Nelson (1992) *Proc. Natl. Acad. Sci. USA* 89, 6639-6643.

Lopata, M. A., Cleveland, D. W., and Sollner-Webb, B. (1984). High-level expression of a chloramphenicol acetyltransferase gene by DEAE-dextran-mediated DNA transfection coupled with a dimethyl sulfoxide or glycerol shock treatment. Nucl. Acids Res. 12, 5707-5717.

Lopez-Corcuera, B and Aragon, C. (1989). Solubilization and reconstitution of the sodium- and chloride-coupled glycine transporter from rat spinal cord. Eur. J. Biochem. 181, 519-524.

Lopez-Corcuera, B., Q.-R. Liu, S. Mandiyan, H. Nelson, and N. Nelson (1992) *J. Biol. Chem.* 267, 17491-17493.

Low, M.J., Lechan, R.M., Hammer, R.E. et al. (1986) Science 231:1002-1004.

Malosio, M.-L., Marqueze-Pouey, B., Kuhse, J., and Betz, H. (1991). Widespread expression of glycine receptor subunit mRNAs in the adult and developing rat brain. EMBO J. 10, 2401-2409

Maniatis, T., Fritsch, E.F., Sambrook, J. (1982) Molecular Cloning, Cold Spring Harbor Laboratory.

Mayor,F., Marvizon, J.G., Aragon, M.C., Gimenez, C., and Valdivieso, F. (1981). Glycine transport into plasma-membrane vesicles derived from rat brain synaptosomes. Biochem. J. 198, 535-541.

Mayser, W., P. Schloss, and H. Betz (1992) *FEBS Letters* 305, 31-36.

Monoghan, D.T. (1990). Glycine modulation of NMDA receptors: Autoradiographic studies; in Glycine

Transmission (Ottersen, O.P. and Storm-Mathisen, J., ed), John Wiley and Sons, New York, pp.219-237.

Moriyoshi, K., Masu, M. Ishii, T., Shigemoto, R., Mizuno, N., Nakanishi, S. (1991). Molecular cloning and characterization of the rat NMDA receptor. Nature 354, 31-37.

Moseley, R., Ballatori, N., and Murphy, S.M. (1988). Na^+-glycine cotransport in canalicular liver plasma membrane vesicles. Am. J. Physiol. 255, G253-259.

Moseley, R., Ballatori, N., and Murphy, S.M. (1988) Am. J. Physiol. 255, G253-259.

Oberdick, J., Smeyne, R.J., Mann, J.R., Jackson, S. and Morgan, J.I. (1990) Science 248:223-226.

Pacholczyk, T., Blakely, R.D., and Amara, S.G. (1991). Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter. Nature 350, 350-354.

Sanger, S. (1977). DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74, 5463-5467.

Sanger, S. (1977) *Proc. Natl. Acad. Sci. USA* 74: 5463-5467.

Shimada, S., Kitayama, S., Lin, C.-L., Patel, A., Nanthakumaar, E., Gregor, P. Kuhar, M. and Uhl, G. (1991). Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. Science 254, 576-578.

Smith, K.E., L.A. Borden, P.R. Hartig, T.A. Branchek, and

R.L. Weinshank (1992) *Neuron* 8, 927-935.

Smith, K.E., L.A. Borden, C.-H.D. Wang, P.R. Hartig, T.A. Branchek, and R.L. Weinshank (1992a) *Mol. Pharm.* 42, 563-569.

Uchida, S., H. M. Kwon, A. Yamauchi, A.S. Preston, F. Marumo, and J. Handler (1992) *Proc. Natl. Acad. Sci. USA* 89, 8230-8234.

van den Pol, A.N. and Gorcs, T. (1988). Glycine and glycine receptor immunoreactivity in brain and spinal cord. J. Neuroscience 8, 472-492.

Weintraub, H.M. (1990) Sci. Am. January p. 40.

Yamauchi, A., S. Uchida, H.M. Kwon, A.S. Preston, R.B. Robey, A. Garcia-Perez, M.B. Burg, and J.S. Handler (1992) *J. Biol. Chem.* 267, 649-652.

Zafra, F. and Gimenez, C. (1986) Br. Res. 397, 108-116.

Zafra, F. and Gimenez, C. (1989). Characteristics and adaptive regulation of glycine transport in cultured glial cells. Biochem. J. 258, 403-408.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Smith, Kelli
Borden, Laurence A.
Branchek, Theresa
Hartig, Paul R.
Weinshank, Richard L.

(ii) TITLE OF INVENTION: DNA ENCODING A GLYCINE TRANSPORTER AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Cooper & Dunham
(B) STREET: 30 Rockefeller Plaza
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: U.S.A.
(F) ZIP: 10112

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.24

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: White, John P.
(B) REGISTRATION NUMBER: 28,678
(C) REFERENCE/DOCKET NUMBER: 1795/39875-A-PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 977-9550
(B) TELEFAX: (212)977-9809
(C) TELEX: 422523 COOP UI

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2121 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(vi) ORIGINAL SOURCE:
(A) ORGANISM: RAT GLYCINE TRANSPORTER

(G) CELL TYPE: MAMMALIAN
(H) CELL LINE: COS7

(vii) IMMEDIATE SOURCE:
(B) CLONE: rB20a

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 62..1975
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GGAGCTGGCA GAGGTGTGAA TGAGCGGCTG AGACACTCGT GCTTTGAGTG CTCTTCCCAG      60

G ATG GCT GTG GCT CAC GGA CCT GTG GCC ACC TCT TCC CCA GAA CAG          106
  Met Ala Val Ala His Gly Pro Val Ala Thr Ser Ser Pro Glu Gln
    1               5                  10                  15

AAT GGT GCT GTG CCC AGC GAG GCC ACC AAG AAG GAC CAG AAC CTC ACA       154
Asn Gly Ala Val Pro Ser Glu Ala Thr Lys Lys Asp Gln Asn Leu Thr
                20                  25                  30

CGG GGC AAC TGG GGC AAC CAG ATC GAG TTT GTA CTG ACG AGC GTG GGC       202
Arg Gly Asn Trp Gly Asn Gln Ile Glu Phe Val Leu Thr Ser Val Gly
            35                  40                  45

TAT GCC GTG GGC CTG GGC AAT GTG TGG CGT TTC CCA TAC CTC TGC TAT       250
Tyr Ala Val Gly Leu Gly Asn Val Trp Arg Phe Pro Tyr Leu Cys Tyr
        50                  55                  60

CGC AAC GGG GGA GGC GCC TTC ATG TTT CCC TAC TTC ATC ATG CTG GTC       298
Arg Asn Gly Gly Gly Ala Phe Met Phe Pro Tyr Phe Ile Met Leu Val
    65                  70                  75

TTC TGC GGC ATT CCT CTC TTC TTC ATG GAG CTC TCC TTC GGC CAG TTT       346
Phe Cys Gly Ile Pro Leu Phe Phe Met Glu Leu Ser Phe Gly Gln Phe
80                  85                  90                  95

GCA AGC CAG GGC TGC CTG GGG GTC TGG AGG ATC AGC CCC ATG TTC AAA       394
Ala Ser Gln Gly Cys Leu Gly Val Trp Arg Ile Ser Pro Met Phe Lys
                100                 105                 110

GGC GTG GGC TAT GGT ATG ATG GTG GTG TCC ACG TAC ATC GGT ATC TAC       442
Gly Val Gly Tyr Gly Met Met Val Val Ser Thr Tyr Ile Gly Ile Tyr
            115                 120                 125

TAC AAC GTG GTC ATC TGC ATC GCC TTC TAC TAC TTC TTC TCG TCC ATG       490
Tyr Asn Val Val Ile Cys Ile Ala Phe Tyr Tyr Phe Phe Ser Ser Met
        130                 135                 140

ACG CAT GTG CTG CCC TGG GCT TAC TGC AAT AAT CCC TGG AAC ACA CCC       538
Thr His Val Leu Pro Trp Ala Tyr Cys Asn Asn Pro Trp Asn Thr Pro
    145                 150                 155

GAC TGT GCC GGT GTG CTG GAT GCT TCC AAT CTC ACC AAT GGC TCC CGG       586
Asp Cys Ala Gly Val Leu Asp Ala Ser Asn Leu Thr Asn Gly Ser Arg
160                 165                 170                 175
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CCC ACT GCC CTG TCT GGC AAC CTG TCT CAC CTG TTC AAC TAC ACC TTG                634
Pro Thr Ala Leu Ser Gly Asn Leu Ser His Leu Phe Asn Tyr Thr Leu
                180                 185                 190

CAA AGG ACC AGC CCC AGT GAG GAG TAC TGG AGG CTG TAT GTG CTG AAG                682
Gln Arg Thr Ser Pro Ser Glu Glu Tyr Trp Arg Leu Tyr Val Leu Lys
            195                 200                 205

CTG TCG GAT GAC ATT GGA GAT TTT GGA GAA GTG CGG CTT CCT CTC CTA                730
Leu Ser Asp Asp Ile Gly Asp Phe Gly Glu Val Arg Leu Pro Leu Leu
        210                 215                 220

GGC TGC CTT GGC GTC TCC TGG GTG GTT GTC TTC CTC TGC CTC ATT CGA                778
Gly Cys Leu Gly Val Ser Trp Val Val Val Phe Leu Cys Leu Ile Arg
    225                 230                 235

GGA GTC AAG TCT TCA GGG AAA GTG GTG TAC TTC ACG GCC ACA TTT CCC                826
Gly Val Lys Ser Ser Gly Lys Val Val Tyr Phe Thr Ala Thr Phe Pro
240                 245                 250                 255

TAT GTG GTG CTG ACC ATT CTG TTT GTT CGT GGA GTG ACC CTG GAA GGA                874
Tyr Val Val Leu Thr Ile Leu Phe Val Arg Gly Val Thr Leu Glu Gly
                260                 265                 270

GCC TTC ACG GGT ATC ATG TAC TAC CTG ACC CCA AAG TGG GAC AAG ATC                922
Ala Phe Thr Gly Ile Met Tyr Tyr Leu Thr Pro Lys Trp Asp Lys Ile
            275                 280                 285

CTG GAG GCC AAG GTG TGG GGG GAT GCA GCC TCT CAG ATC TTC TAT TCC                970
Leu Glu Ala Lys Val Trp Gly Asp Ala Ala Ser Gln Ile Phe Tyr Ser
        290                 295                 300

CTG GGC TGT GCA TGG GGT GGC CTC ATC ACC ATG GCA TCC TAC AAC AAA               1018
Leu Gly Cys Ala Trp Gly Gly Leu Ile Thr Met Ala Ser Tyr Asn Lys
    305                 310                 315

TTC CAC AAC AAC TGC TAC CGG GAC AGC GTC ATC ATC AGC ATC ACC AAT               1066
Phe His Asn Asn Cys Tyr Arg Asp Ser Val Ile Ile Ser Ile Thr Asn
320                 325                 330                 335

TGT GCT ACC AGT GTC TAT GCT GGC TTC GTC ATC TTC TCT ATC CTA GGC               1114
Cys Ala Thr Ser Val Tyr Ala Gly Phe Val Ile Phe Ser Ile Leu Gly
                340                 345                 350

TTC ATG GCC AAT CAC CTG GGT GTG GAT GTG TCT CGG GTG GCA GAC CAC               1162
Phe Met Ala Asn His Leu Gly Val Asp Val Ser Arg Val Ala Asp His
            355                 360                 365

GGG CCC GGG CTA GCT TTC GTG GCT TAC CCC GAG GCT CTC ACA CTG CTT               1210
Gly Pro Gly Leu Ala Phe Val Ala Tyr Pro Glu Ala Leu Thr Leu Leu
        370                 375                 380

CCC ATC TCC CCG CTC TGG TCC TTG CTG TTT TTC TTC ATG CTC ATC CTG               1258
Pro Ile Ser Pro Leu Trp Ser Leu Leu Phe Phe Phe Met Leu Ile Leu
    385                 390                 395

CTG GGA CTC GGT ACT CAG TTC TGC CTC CTG GAG ACC CTA GTC ACT GCC               1306
Leu Gly Leu Gly Thr Gln Phe Cys Leu Leu Glu Thr Leu Val Thr Ala
400                 405                 410                 415
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ATT GTG GAT GAG GTG GGG AAT GAG TGG ATT CTG CAG AAG AAG ACC TAC      1354
Ile Val Asp Glu Val Gly Asn Glu Trp Ile Leu Gln Lys Lys Thr Tyr
                420                 425                 430

GTG ACC TTG GGT GTG GCT GTG GCT GGC TTC TTG CTG GGT ATC CCT CTT      1402
Val Thr Leu Gly Val Ala Val Ala Gly Phe Leu Leu Gly Ile Pro Leu
            435                 440                 445

ACC AGC CAG GCG GGC ATC TAC TGG CTG CTG TTG ATG GAC AAC TAC GCA      1450
Thr Ser Gln Ala Gly Ile Tyr Trp Leu Leu Leu Met Asp Asn Tyr Ala
        450                 455                 460

GCC AGC TTC TCC TTG GTT GTC ATC TCC TGC ATC ATG TGC GTG TCC ATC      1498
Ala Ser Phe Ser Leu Val Val Ile Ser Cys Ile Met Cys Val Ser Ile
    465                 470                 475

ATG TAT ATC TAT GGG CAC CGG AAC TAC TTC CAG GAC ATT CAG ATG ATG      1546
Met Tyr Ile Tyr Gly His Arg Asn Tyr Phe Gln Asp Ile Gln Met Met
480                 485                 490                 495

CTG GGG TTC CCA CCG CCT CTC TTC TTC CAG ATC TGT TGG CGT TTT GTC      1594
Leu Gly Phe Pro Pro Pro Leu Phe Phe Gln Ile Cys Trp Arg Phe Val
                500                 505                 510

TCT CCC ACT ATC ATC TTT TTC ATT CTC ATC TTC ACG GTG ATC CAG TAC      1642
Ser Pro Thr Ile Ile Phe Phe Ile Leu Ile Phe Thr Val Ile Gln Tyr
            515                 520                 525

CGG CCA ATC ACT TAC AAC CAC TAC CAG TAC CCA GGC TGG GCT GTG GCC      1690
Arg Pro Ile Thr Tyr Asn His Tyr Gln Tyr Pro Gly Trp Ala Val Ala
        530                 535                 540

ATC GGC TTC CTC ATG GCT TTG TCG TCT GTC ATC TGC ATC CCA TTG TAC      1738
Ile Gly Phe Leu Met Ala Leu Ser Ser Val Ile Cys Ile Pro Leu Tyr
    545                 550                 555

GCA TTG TTC CAG CTC TGC CGC ACA GAT GGG GAC ACA CTT CTT CAG CGT      1786
Ala Leu Phe Gln Leu Cys Arg Thr Asp Gly Asp Thr Leu Leu Gln Arg
560                 565                 570                 575

TTG AAA AAT GCC ACA AAG CCA AGC AGA GAC TGG GGC CCT GCC CTC CTG      1834
Leu Lys Asn Ala Thr Lys Pro Ser Arg Asp Trp Gly Pro Ala Leu Leu
                580                 585                 590

GAG CAC CGG ACT GGG CGC TAT GCC CCC ACT ACA ACC CCC TCT CCT GAA      1882
Glu His Arg Thr Gly Arg Tyr Ala Pro Thr Thr Thr Pro Ser Pro Glu
            595                 600                 605

GAT GGG TTT GAG GTT CAG CCA CTG CAC CCG GAC AAG GCC CAG ATC CCC      1930
Asp Gly Phe Glu Val Gln Pro Leu His Pro Asp Lys Ala Gln Ile Pro
        610                 615                 620

ATC GTG GGC AGT AAC GGC TCC AGC CGC CTC CAG GAC TCC CGG ATA          1975
Ile Val Gly Ser Asn Gly Ser Ser Arg Leu Gln Asp Ser Arg Ile
    625                 630                 635

TGAGCACAGT TGTTGCAAGG GGAGAAGCCC CACCCAACCC TTGCTCCTAC CACAGAGACT    2035

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GTCACCTTGG CCACCACTGC TCATGT                                         2121
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 638 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Val Ala His Gly Pro Val Ala Thr Ser Ser Pro Glu Gln Asn
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Gly Ala Val Pro Ser Glu Ala Thr Lys Lys Asp Gln Asn Leu Thr Arg
20 25 30

Gly Asn Trp Gly Asn Gln Ile Glu Phe Val Leu Thr Ser Val Gly Tyr
35 40 45

Ala Val Gly Leu Gly Asn Val Trp Arg Phe Pro Tyr Leu Cys Tyr Arg
50 55 60

Asn Gly Gly Gly Ala Phe Met Phe Pro Tyr Phe Ile Met Leu Val Phe
65 70 75 80

Cys Gly Ile Pro Leu Phe Phe Met Glu Leu Ser Phe Gly Gln Phe Ala
85 90 95

Ser Gln Gly Cys Leu Gly Val Trp Arg Ile Ser Pro Met Phe Lys Gly
100 105 110

Val Gly Tyr Gly Met Met Val Val Ser Thr Tyr Ile Gly Ile Tyr Tyr
115 120 125

Asn Val Val Ile Cys Ile Ala Phe Tyr Tyr Phe Phe Ser Ser Met Thr
130 135 140

His Val Leu Pro Trp Ala Tyr Cys Asn Asn Pro Trp Asn Thr Pro Asp
145 150 155 160

Cys Ala Gly Val Leu Asp Ala Ser Asn Leu Thr Asn Gly Ser Arg Pro
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Thr Ala Leu Ser Gly Asn Leu Ser His Leu Phe Asn Tyr Thr Leu Gln
180 185 190

Arg Thr Ser Pro Ser Glu Glu Tyr Trp Arg Leu Tyr Val Leu Lys Leu
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Ser Asp Asp Ile Gly Asp Phe Gly Glu Val Arg Leu Pro Leu Leu Gly
210 215 220

Cys Leu Gly Val Ser Trp Val Val Val Phe Leu Cys Leu Ile Arg Gly
225 230 235 240

Val Lys Ser Ser Gly Lys Val Val Tyr Phe Thr Ala Thr Phe Pro Tyr
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Phe Thr Gly Ile Met Tyr Tyr Leu Thr Pro Lys Trp Asp Lys Ile Leu
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Glu Ala Lys Val Trp Gly Asp Ala Ala Ser Gln Ile Phe Tyr Ser Leu
290 295 300

Gly Cys Ala Trp Gly Gly Leu Ile Thr Met Ala Ser Tyr Asn Lys Phe
305 310 315 320

His Asn Asn Cys Tyr Arg Asp Ser Val Ile Ile Ser Ile Thr Asn Cys
325 330 335

Ala Thr Ser Val Tyr Ala Gly Phe Val Ile Phe Ser Ile Leu Gly Phe
340 345 350

Met Ala Asn His Leu Gly Val Asp Val Ser Arg Val Ala Asp His Gly
355 360 365

Pro Gly Leu Ala Phe Val Ala Tyr Pro Glu Ala Leu Thr Leu Leu Pro
370 375 380

Ile Ser Pro Leu Trp Ser Leu Leu Phe Phe Phe Met Leu Ile Leu Leu
385 390 395 400

Gly Leu Gly Thr Gln Phe Cys Leu Leu Glu Thr Leu Val Thr Ala Ile
405 410 415

Val Asp Glu Val Gly Asn Glu Trp Ile Leu Gln Lys Lys Thr Tyr Val
420 425 430

Thr Leu Gly Val Ala Val Ala Gly Phe Leu Leu Gly Ile Pro Leu Thr
435 440 445

Ser Gln Ala Gly Ile Tyr Trp Leu Leu Leu Met Asp Asn Tyr Ala Ala
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Ser Phe Ser Leu Val Val Ile Ser Cys Ile Met Cys Val Ser Ile Met
465 470 475 480

Tyr Ile Tyr Gly His Arg Asn Tyr Phe Gln Asp Ile Gln Met Met Leu
485 490 495

Gly Phe Pro Pro Pro Leu Phe Phe Gln Ile Cys Trp Arg Phe Val Ser
500 505 510

Pro Thr Ile Ile Phe Phe Ile Leu Ile Phe Thr Val Ile Gln Tyr Arg
515 520 525

Pro Ile Thr Tyr Asn His Tyr Gln Tyr Pro Gly Trp Ala Val Ala Ile
530 535 540

Gly Phe Leu Met Ala Leu Ser Ser Val Ile Cys Ile Pro Leu Tyr Ala
545 550 555 560

Leu Phe Gln Leu Cys Arg Thr Asp Gly Asp Thr Leu Leu Gln Arg Leu
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Lys Asn Ala Thr Lys Pro Ser Arg Asp Trp Gly Pro Ala Leu Leu Glu
580 585 590

His Arg Thr Gly Arg Tyr Ala Pro Thr Thr Thr Pro Ser Pro Glu Asp
595 600 605

Gly Phe Glu Val Gln Pro Leu His Pro Asp Lys Ala Gln Ile Pro Ile
610 615 620

Val Gly Ser Asn Gly Ser Ser Arg Leu Gln Asp Ser Arg Ile
625 630 635

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 617 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: N

(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: HUMAN NORADRENALINE TRANSPORTER

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Leu Leu Ala Arg Met Asn Pro Gln Val Gln Pro Glu Asn Asn Gly
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Leu Leu Val Val Lys Glu Arg Asn Gly Val Gln Cys Leu Leu Ala Pro
35 40 45

Arg Asp Gly Asp Ala Gln Pro Arg Glu Thr Trp Gly Lys Lys Ile Asp
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Phe Leu Leu Ser Val Val Gly Phe Ala Val Asp Leu Ala Asn Val Trp
65 70 75 80

Arg Phe Pro Tyr Leu Cys Tyr Lys Asn Gly Gly Gly Ala Phe Leu Ile
85 90 95

Pro Tyr Thr Leu Phe Leu Ile Ile Ala Gly Met Pro Leu Phe Tyr Met
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Glu Leu Ala Leu Gly Gln Tyr Asn Arg Glu Gly Ala Ala Thr Val Trp
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Lys Ile Cys Pro Phe Phe Lys Gly Val Gly Tyr Ala Val Ile Leu Ile
130 135 140

Ala Leu Tyr Val Gly Phe Tyr Tyr Asn Val Ile Ile Ala Trp Ser Leu
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Tyr Tyr Leu Phe Ser Ser Phe Thr Leu Asn Leu Pro Trp Thr Asp Cys
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Gly His Thr Trp Asn Ser Pro Asn Cys Thr Asp Pro Lys Leu Leu Asn
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Gly Ser Val Leu Gly Asn His Thr Lys Tyr Ser Lys Tyr Lys Phe Thr
195 200 205

Pro Ala Ala Glu Phe Tyr Glu Arg Gly Val Leu His Leu His Glu Ser
210 215 220

Ser Gly Ile His Asp Ile Gly Leu Pro Gln Trp Gln Leu Leu Leu Cys
225 230 235 240

Leu Met Val Val Val Ile Val Leu Tyr Phe Ser Leu Trp Lys Gly Val
245 250 255

Lys Thr Ser Gly Lys Val Val Trp Ile Thr Ala Thr Leu Pro Tyr Phe
260 265 270

Val Leu Phe Val Leu Leu Val His Gly Val Thr Leu Pro Gly Ala Ser
275 280 285

Asn Gly Ile Asn Ala Tyr Leu His Ile Asp Phe Tyr Arg Leu Lys Glu
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Ala Thr Val Trp Ile Asp Ala Ala Thr Gln Ile Phe Phe Ser Leu Gly
305 310 315 320

Ala Gly Phe Gly Val Leu Ile Ala Phe Ala Ser Tyr Asn Lys Phe Asp
325 330 335

Asn Asn Cys Tyr Arg Asp Ala Leu Leu Thr Ser Ser Ile Asn Cys Ile
340 345 350

Thr Ser Phe Val Ser Gly Phe Ala Ile Phe Ser Ile Leu Gly Tyr Met
355 360 365

Ala His Glu His Lys Val Asn Ile Glu Asp Val Ala Thr Glu Gly Ala
370 375 380

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405 410 415

Leu Asp Ser Ser Met Gly Gly Met Glu Ala Val Ile Thr Gly Leu Ala
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Asp Asp Phe Gln Val Leu Lys Arg His Arg Lys Leu Phe Thr Phe Gly
435 440 445

Val Thr Phe Ser Thr Phe Leu Leu Ala Leu Phe Cys Ile Thr Lys Gly
450 455 460

Gly Ile Tyr Val Leu Thr Leu Leu Asp Thr Phe Ala Ala Gly Thr Ser
465 470 475 480

Ile Leu Phe Ala Val Leu Met Glu Ala Ile Gly Val Ser Trp Phe Tyr
485 490 495

Gly Val Asp Arg Phe Ser Asn Asp Ile Gln Gln Met Met Gly Phe Arg
500 505 510

Pro Gly Leu Tyr Trp Arg Leu Cys Trp Lys Phe Val Ser Pro Ala Phe
515 520 525

Leu Leu Phe Val Val Val Val Ser Ile Ile Asn Phe Lys Pro Leu Thr
530 535 540

Tyr Asp Asp Tyr Ile Phe Pro Pro Trp Ala Asn Trp Val Gly Trp Gly
545 550 555 560

Ile Ala Leu Ser Ser Met Val Leu Val Pro Ile Tyr Val Ile Tyr Lys
565 570 575

Phe Leu Ser Thr Gln Gly Ser Leu Trp Glu Arg Leu Ala Tyr Gly Ile
580 585 590

Thr Pro Glu Asn Glu His His Leu Val Ala Gln Arg Asp Ile Arg Gln
595 600 605

Phe Gln Leu Gln His Trp Leu Ala Ile
610 615

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 599 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: N

(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: RAT GABA TRANSPORTER (GAT-1)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Thr Asp Asn Ser Lys Val Ala Asp Gly Gln Ile Ser Thr Glu
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Lys Val Gln Lys Lys Ala Gly Asp Leu Pro Asp Arg Asp Thr Trp Lys
35 40 45

Gly Arg Phe Asp Phe Leu Met Ser Cys Val Gly Tyr Ala Ile Gly Leu
50 55 60

Gly Asn Val Trp Arg Phe Pro Tyr Leu Cys Gly Lys Asn Gly Gly Gly
65 70 75 80

Ala Phe Leu Ile Pro Tyr Phe Leu Thr Leu Ile Phe Ala Gly Val Pro
85 90 95

Leu Phe Leu Leu Glu Cys Ser Leu Gly Gln Tyr Thr Ser Ile Gly Gly
100 105 110

Leu Gly Val Trp Lys Leu Ala Pro Met Phe Lys Gly Val Gly Leu Ala
115 120 125

Ala Ala Val Leu Ser Phe Trp Leu Asn Ile Tyr Tyr Ile Val Ile Ile
130 135 140

Ser Trp Ala Ile Tyr Tyr Leu Tyr Asn Ser Phe Thr Thr Thr Leu Pro
145 150 155 160

Trp Lys Gln Cys Asp Asn Pro Trp Asn Thr Asp Arg Cys Phe Ser Asn
165 170 175

Tyr Ser Leu Val Asn Thr Thr Asn Met Thr Ser Ala Val Val Glu Phe
180 185 190

Trp Glu Arg Asn Met His Gln Met Thr Asp Gly Leu Asp Lys Pro Gly
195 200 205

Gln Ile Arg Trp Pro Leu Ala Ile Thr Leu Ala Ile Ala Trp Val Leu
210 215 220

Val Tyr Phe Cys Ile Trp Lys Gly Val Gly Trp Thr Gly Lys Val Val
225 230 235 240

Tyr Phe Ser Ala Thr Tyr Pro Tyr Ile Met Leu Ile Ile Leu Phe Phe
245 250 255

Arg Gly Val Thr Leu Pro Gly Ala Lys Glu Gly Ile Leu Phe Tyr Ile
260 265 270

Thr Pro Asn Phe Arg Lys Leu Ser Asp Ser Glu Val Trp Leu Asp Ala
275 280 285

Ala Thr Gln Ile Phe Phe Ser Tyr Gly Leu Gly Leu Gly Ser Leu Ile
290 295 300

Ala Leu Gly Ser Tyr Asn Ser Phe His Asn Asn Val Tyr Arg Asp Ser
305 310 315 320

Ile Ile Val Cys Cys Ile Asn Ser Cys Thr Ser Met Phe Ala Gly Phe
325 330 335

Val Ile Phe Ser Ile Val Gly Phe Met Ala His Val Thr Lys Arg Ser
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Ile Ala Asp Val Ala Ala Ser Gly Pro Gly Leu Ala Phe Leu Ala Tyr
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Pro Glu Ala Val Thr Gln Leu Pro Ile Ser Pro Leu Trp Ala Ile Leu
370 375 380

Phe Phe Ser Met Leu Leu Met Leu Gly Ile Asp Ser Gln Phe Cys Thr
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Val Glu Gly Phe Ile Thr Ala Leu Val Asp Glu Tyr Pro Arg Leu Leu
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Leu Phe Asp Tyr Tyr Ser Ala Ser Gly Met Ser Leu Leu Phe Leu Val
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Leu Phe Ser Ala Val Gln Met Thr Pro Leu Thr Met Gly Ser Tyr Val
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Met Val Leu Ile Pro Gly Tyr Met Ala Tyr Met Phe Leu Thr Leu Lys
545 550 555 560

Gly Ser Leu Lys Gln Arg Leu Gln Val Met Ile Gln Pro Ser Glu Asp
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580 585 590

Ala Ser Lys Glu Ala Tyr Ile
595

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 981 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: N

(iv) ANTI-SENSE: N

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HUMAN GLYCINE TRANSPORTER

(vii) IMMEDIATE SOURCE:
(B) CLONE: pBluescript-hTC27a

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 46..981
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GGCAGGGGAT GCGTCAGTGT CGCGCTGGAG CTGGCAGAGG TGTGA ATG AGC GGC 54
Met Ser Gly
1

GGA GAC ACG CGG GCT GCG ATC GCT CGC CCC AGG ATG GCC GCG GCT CAT 102
Gly Asp Thr Arg Ala Ala Ile Ala Arg Pro Arg Met Ala Ala Ala His
5 10 15

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GGA CCT GTG GCC CCC TCT TCC CCA GAA CAG AAT GGT GCT GTG CCC AGC          150
Gly Pro Val Ala Pro Ser Ser Pro Glu Gln Asn Gly Ala Val Pro Ser
 20                  25                  30                  35

GAG GCC ACC AAG AGG GAC CAG AAC CTC AAA CGG GGC AAC TGG GGC AAC          198
Glu Ala Thr Lys Arg Asp Gln Asn Leu Lys Arg Gly Asn Trp Gly Asn
                 40                  45                  50

CAG ATC GAG TTT GTA CTG ACG AGC GTG GGC TAT GCC GTG GGC CTG GGC          246
Gln Ile Glu Phe Val Leu Thr Ser Val Gly Tyr Ala Val Gly Leu Gly
             55                  60                  65

AAT GTC TGG CGC TTC CCA TAC CTC TGC TAT CGC AAC GGG GGA GGC GCC          294
Asn Val Trp Arg Phe Pro Tyr Leu Cys Tyr Arg Asn Gly Gly Gly Ala
         70                  75                  80

TTC ATG TTC CCC TAC TTC ATC ATG CTC ATC TTC TGC GGG ATC CCC CTC          342
Phe Met Phe Pro Tyr Phe Ile Met Leu Ile Phe Cys Gly Ile Pro Leu
     85                  90                  95

TTC TTC ATG GAG CTC TCC TTC GGC CAG TTT GCA AGC CAG GGG TGC CTG          390
Phe Phe Met Glu Leu Ser Phe Gly Gln Phe Ala Ser Gln Gly Cys Leu
100                 105                 110                 115

GGG GTC TGG AGG ATC AGC CCC ATG TTC AAA GGA GTG GGC TAT GGT ATG          438
Gly Val Trp Arg Ile Ser Pro Met Phe Lys Gly Val Gly Tyr Gly Met
                120                 125                 130

ATG GTG GTG TCC ACC TAC ATC GGC ATC TAC TAC AAT GTG GTC ATC TGC          486
Met Val Val Ser Thr Tyr Ile Gly Ile Tyr Tyr Asn Val Val Ile Cys
            135                 140                 145

ATC GCC TTC TAC TAC TTC TTC TCG TCC ATG ACG CAC GTG CTG CCC TGG          534
Ile Ala Phe Tyr Tyr Phe Phe Ser Ser Met Thr His Val Leu Pro Trp
        150                 155                 160

GCC TAC TGC AAT AAC CCC TGG AAC ACG CAT GAC TGC GCC GGT GTA CTG          582
Ala Tyr Cys Asn Asn Pro Trp Asn Thr His Asp Cys Ala Gly Val Leu
    165                 170                 175

GAC GCC TCC AAC CTC ACC AAT GGC TCT CGG CCA GCC GCC TTG CCC AGC          630
Asp Ala Ser Asn Leu Thr Asn Gly Ser Arg Pro Ala Ala Leu Pro Ser
180                 185                 190                 195

AAC CTC TCC CAC CTG CTC AAC CAC AGC CTC CAG AGG ACC AGC CCC AGC          678
Asn Leu Ser His Leu Leu Asn His Ser Leu Gln Arg Thr Ser Pro Ser
                200                 205                 210

GAG GAG TAC TGG AGG CTG TAC GTG CTG AAG CTG TCA GAT GAC ATT GGG          726
Glu Glu Tyr Trp Arg Leu Tyr Val Leu Lys Leu Ser Asp Asp Ile Gly
            215                 220                 225

AAC TTT GGG GAG GTG CGG CTG CCC CTC CTT GGC TGC CTC GGT GTC TCC          774
Asn Phe Gly Glu Val Arg Leu Pro Leu Leu Gly Cys Leu Gly Val Ser
        230                 235                 240

TGG TTG GTC GTC TTC CTC TGC CTC ATC CGA GGG GTC AAG TCT TCA GGG          822
Trp Leu Val Val Phe Leu Cys Leu Ile Arg Gly Val Lys Ser Ser Gly
    245                 250                 255
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AAA GTG GTG TAC TTC ACG GCC ACG TTC CCC TAC GTG GTG CTG ACC ATT      870
Lys Val Val Tyr Phe Thr Ala Thr Phe Pro Tyr Val Val Leu Thr Ile
260                 265                 270                 275

CTG TTT GTC CGC GGA GTG ACC CTG GAG GGA GCC TTT GAC GGC ATC ATG      918
Leu Phe Val Arg Gly Val Thr Leu Glu Gly Ala Phe Asp Gly Ile Met
                280                 285                 290

TAC TAC CTA ACC CCG CAG TGG GAC AAG ATC CTG GAG GCC AAG GTG TGG      966
Tyr Tyr Leu Thr Pro Gln Trp Asp Lys Ile Leu Glu Ala Lys Val Trp
            295                 300                 305

                                                                     981
GGT GAT GCT GCC TCC
Gly Asp Ala Ala Ser
        310
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 312 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Ser Gly Gly Asp Thr Arg Ala Ala Ile Ala Arg Pro Arg Met Ala
 1               5                  10                  15

Ala Ala His Gly Pro Val Ala Pro Ser Ser Pro Glu Gln Asn Gly Ala
            20                  25                  30

Val Pro Ser Glu Ala Thr Lys Arg Asp Gln Asn Leu Lys Arg Gly Asn
        35                  40                  45

Trp Gly Asn Gln Ile Glu Phe Val Leu Thr Ser Val Gly Tyr Ala Val
    50                  55                  60

Gly Leu Gly Asn Val Trp Arg Phe Pro Tyr Leu Cys Tyr Arg Asn Gly
65                  70                  75                  80

Gly Gly Ala Phe Met Phe Pro Tyr Phe Ile Met Leu Ile Phe Cys Gly
                85                  90                  95

Ile Pro Leu Phe Phe Met Glu Leu Ser Phe Gly Gln Phe Ala Ser Gln
            100                 105                 110

Gly Cys Leu Gly Val Trp Arg Ile Ser Pro Met Phe Lys Gly Val Gly
        115                 120                 125

Tyr Gly Met Met Val Val Ser Thr Tyr Ile Gly Ile Tyr Tyr Asn Val
    130                 135                 140

Val Ile Cys Ile Ala Phe Tyr Tyr Phe Phe Ser Ser Met Thr His Val
145                 150                 155                 160

Leu Pro Trp Ala Tyr Cys Asn Asn Pro Trp Asn Thr His Asp Cys Ala
                165                 170                 175
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Gly Val Leu Asp Ala Ser Asn Leu Thr Asn Gly Ser Arg Pro Ala Ala
180 185 190

Leu Pro Ser Asn Leu Ser His Leu Leu Asn His Ser Leu Gln Arg Thr
195 200 205

Ser Pro Ser Glu Glu Tyr Trp Arg Leu Tyr Val Leu Lys Leu Ser Asp
210 215 220

Asp Ile Gly Asn Phe Gly Glu Val Arg Leu Pro Leu Leu Gly Cys Leu
225 230 235 240

Gly Val Ser Trp Leu Val Val Phe Leu Cys Leu Ile Arg Gly Val Lys
245 250 255

Ser Ser Gly Lys Val Val Tyr Phe Thr Ala Thr Phe Pro Tyr Val Val
260 265 270

Leu Thr Ile Leu Phe Val Arg Gly Val Thr Leu Glu Gly Ala Phe Asp
275 280 285

Gly Ile Met Tyr Tyr Leu Thr Pro Gln Trp Asp Lys Ile Leu Glu Ala
290 295 300

Lys Val Trp Gly Asp Ala Ala Ser
305 310

What is claimed is:

1. An isolated nucleic acid molecule encoding a mammalian glycine transporter.

2. A nucleic acid molecule of claim 1, wherein the nucleic acid molecule encodes a human glycine transporter.

3. An isolated nucleic acid molecule of claim 1, wherein the nucleic acid molecule is a DNA molecule.

4. An isolated DNA molecule of claim 3, wherein the DNA molecule is a cDNA molecule.

5. A DNA molecule of claim 3 wherein the DNA molecule is derived from genomic DNA.

6. A isolated nucleic acid molecule which has a nucleic acid sequence which differs from the sequence of a nucleic acid molecule encoding a glycine transporter at one or more nucleotides and which does not encode a protein having glycine transporter activity.

7. A nucleic acid molecule of claim 6, wherein the nucleic acid molecule is a DNA molecule.

8. A DNA molecule of claim 7, wherein the DNA molecule is a cDNA molecule.

9. A vector comprising a DNA molecule of claim 3.

10. A plasmid vector of claim 9.

11. A vector of claim 9 adapted for expression in a bacterial cell which comprises the regulatory elements necessary for expression of the DNA encoding a glycine transporter in the bacterial cell so located relative to the DNA as to permit expression thereof.

12. A vector of claim 9 adapted for expression in a yeast cell which comprises the regulatory elements necessary for the expression of the DNA encoding a glycine transporter in the yeast cell so located relative to the DNA as to permit expression thereof.

13. A vector of claim 9 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA encoding a glycine transporter in the mammalian cell so located relative to the DNA as to permit expression thereof.

14. A plasmid of claim 10 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the DNA in the mammalian cell so located relative to the DNA encoding a glycine transporter as to permit expression thereof.

15. A plasmid of claim 14 designated pSVL-rB20a (ATCC Accession No.75132).

16. A plasmid of claim 14 designated pBluescript-hTC27a (ATCC Accession No.).

17. A mammalian cell comprising the plasmid of claim 10.

18. The mammalian cell of claim 17, wherein the mammalian cell is a Cos7 cell.

19. A Cos7 cell comprising the plasmid of claim 15.

20. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a mammalian glycine transporter.

21. A nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding a human glycine transporter.

22. The nucleic acid probe of claim 20, wherein the nucleic acid is DNA.

23. A mixture of nucleic acid probes in accordance with claim 20, such probes having sequences which differ from one another at predefined positions.

24. An antisense oligonucleotide having a sequence capable of specifically binding to a mRNA molecule encoding a mammalian glycine transporter so as to prevent translation of the mRNA molecule.

25. An antisense oligonucleotide capable of specifically binding to a mRNA molecule encoding a human glycine transporter so as to prevent translation of the mRNA molecule.

26. An antisense oligonucleotide of claim 24 comprising chemical analogs of nucleotides.

27. A mixture of antisense oligonucleotides according to claim 24, such oligonucleotides having sequences which differ from one another at predefined positions.

28. A method for detecting expression of a mammalian glycine transporter, which comprises obtaining RNA from cells or tissue, contacting the RNA so obtained with a nucleic acid probe of claim 20 under hybridizing conditions, detecting the presence of any mRNA hybridized to the probe, the presence of mRNA hybridized to the probe indicating expression of the mammalian glycine transporter, and thereby detecting the expression of the mammalian glycine transporter.

29. A method for detecting expression of a human glycine transporter, which comprises obtaining RNA from cells or tissue, contacting the RNA so obtained with a nucleic acid probe of claim 21 under hybridizing conditions, detecting the presence of any mRNA hybridized to the probe, the presence of mRNA hybridized to the probe indicating expression of the human glycine transporter, and thereby detecting the expression of the human glycine transporter.

30. A method of detecting expression of a mammalian glycine transporter in a cell or tissue by in situ hybridization, which comprises contacting the cell or tissue with a nucleic acid probe of claim 20 under hybridizing conditions, detecting the presence of any mRNA hybridized to the probe, the presence of

mRNA hybridized to the probe indicating expression of a mammalian glycine transporter, and thereby detecting the expression of a mammalian glycine transporter .

31. A method of detecting expression of a human glycine transporter in a cell or tissue by in situ hybridization, which comprises contacting the cell or tissue with a nucleic acid probe of claim 21 under hybridizing conditions, detecting the presence of any mRNA hybridized to the probe, the presence of mRNA hybridized to the probe indicating expression of a human glycine transporter, and thereby detecting the expression of the human glycine transporter.

32. A method of isolating from a gene library a gene encoding a transporter other than the glycine transporter which comprises contacting the library under hybridizing conditions with a probe of claim 20 and isolating any gene to which the probe hybridizes.

33. A method of claim 31, which additionally comprises simultaneously contacting the DNA comprising the library under hybridizing conditions with a second nucleic acid probe comprising a sequence capable of hybridizing to a DNA sequence of the complementary strand of the DNA of the gene to which the first probe hybridizes, treating any gene sequence to which both probes hybridized so as to produce multiple copies of the gene sequence, isolating the amplified gene sequence and using the isolated gene sequence as a probe to isolate from a gene library the gene to which the amplified DNA sequence

hybridizes.

34. The gene isolated by the method of claim 32 or 33.

35. A synthetic gene which comprises the isolated nulceic acid molecule of claim 1 and at least one regulatory element attached thereto so as to increase the number of RNA molecules transcribed from the synthetic gene.

36. A synthetic gene which comprises the isolated nulceic acid molecule of claim 1 and at least one regulatory element attached thereto so as to decrease the number of RNA molecules transcribed from the synthetic gene.

37. An isolated mammalian glycine transporter protein.

38. The transporter protein of claim 37, wherein the mammalian glycine transporter protein is a human glycine transporter.

39. A method of preparing a mammalian glycine transporter of claim 37, which comprises inducing cells to express the mammalian glycine transporter and recovering the mammalian glycine transporter from the resulting cells.

40. A method of preparing a mammalian glycine transporter protein of claim 37, which comprises inserting a nucleic acid molecule encoding the mammalian glycine in a suitable vector, inserting the resulting vector in suitable host cell and recovering the mammalian glycine transporter by the resulting cell.

41. A method of preparing a human glycine transporter of claim 38, which comprises inducing cells to express the human glycine transporter and recovering the human glycine transporter from the resulting cells.

42. A method of preparing a human glycine transporter protein of claim 38, which comprises inserting a nucleic acid molecule encoding the human glycine transporter in a suitable vector, inserting the resulting vector in suitable host cell and recovering the human glycine transporter produced by the resulting cell.

43. An antibody directed to a mammalian glycine transporter or to a protein fragment of the mammalian glycine transporter.

44. An antibody directed to a human glycine transporter or to a protein fragment of the human glycine transporter.

45. An antibody of claim 43, wherein the antibody is a monoclonal antibody.

46. An antibody of claim 44, wherein the antibody is a monoclonal antibody.

47. A monoclonal antibody of claim 45, wherein the antibody is directed to an epitope of a mammalian cell-surface glycine transporter and having an amino acid sequence substantially the same as the amino acid sequence of a cell-surface epitope of the mammalian glycine transporter.

48. A monoclonal antibody of claim 46, wherein the antibody is directed to an epitope of a human cell-

surface glycine transporter and having an amino acid sequence substantially the same as the amino acid sequence for a cell-surface epitope of the human glycine transporter.

49. A pharmaceutical composition comprising an amount of a substance effective to alleviate the abnormalities resulting from overexpression of a human glycine transporter and a pharmaceutically acceptable carrier.

50. A pharmaceutical composition comprising an amount of a substance effective to alleviate abnormalities resulting from underexpression of a human glycine transporter and a pharmaceutically acceptable carrier.

51. A pharmaceutical composition comprising an effective amount of an oligonucleotide of claim 25 effective to reduce expression of a human glycine transporter by passing through a cell membrane and specifically binding with mRNA encoding a human glycine transporter in the cell so as to prevent its translation and a pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane.

52. A pharmaceutical composition claim 51, wherein the nucleotide is coupled to a substance which inactivates mRNA.

53. A pharmaceutical composition of claim 52, wherein the substance which inactivates the mRNA is a ribozyme.

54. A pharmaceutical composition of claim 52, wherein the pharmaceutically acceptable hydrophobic carrier capable of passing through a cell membrane comprises a structure which binds to a transporter specific for a selected cell type and is thereby taken up by the cells of the selected cell type.

55. A pharmaceutical composition which comprises an amount of the antibody of claim 44 effective to block binding of naturally occurring substrates to a human glycine transporter and a pharmaceutically acceptable carrier.

56. A transgenic nonhuman mammal which comprises a nucleic acid molecule of claim 1.

57. A transgenic nonhuman mammal which comprises the nucleic acid molecule of claim 6.

58. A transgenic nonhuman mammal whose genome comprises a nucleic acid molecule of claim 1 so placed as to be transcribed into antisense mRNA complementary to mRNA encoding a human glycine transporter and which hybridizes to mRNA encoding a human glycine transporter thereby reducing its translation.

59. The transgenic nonhuman mammal of claim 56, wherein the nucleic acid molecule further comprises an inducible promoter.

60. The transgenic nonhuman mammal of claim 56 or 57 wherein the nucleic molecule additionally comprises tissue specific regulatory elements.

61. The transgenic non-human mammal of 56, wherein the

transgenic non-human mammal is a mouse.

62. A method for determining the physiological effects of varying the levels of expression of a human glycine transporter which comprises producing a transgenic non-human mammal whose levels of expression of a human glycine transporter can be varied by use of an inducible promoter.

63. A method for determining the physiological effects of expressing varying levels of a human glycine transporter which comprises producing a panel of transgenic non-human mammals each expressing a different amount of a human glycine transporter.

64. A method for determining whether a compound not known to be capable of specifically binding to a human glycine transporter can specifically bind to the human glycine transporter, which comprises contacting a mammalian cell comprising a plasmid adapted for expression in a mammalian cell which plasmid further comprises a DNA which expresses a human glycine transporter on the cell's surface with the compound under conditions permitting binding of ligands known to bind to a human glycine transporter, detecting the presence of any compound bound to the human glycine transporter, the presence of bound compound indicating that the compound is capable of specifically binding to the human glycine transporter.

65. The method of claim 64, wherein the mammalian cell is a non-neuronal cell.

66. The method of claim 65, wherein the non-neuronal

cell is a COS7 cell.

67. A method of screening compounds to identify drugs which interact with, and specifically bind to, a human glycine transporter on the surface of a cell, which comprises contacting a mammalian cell which comprises a plasmid adapted for expression in a mammalian cell which plasmid further comprises DNA which expresses a human glycine transporter on the cell's surface with a plurality of compounds, determining those compounds which bind to the human glycine transporter expressed on the cell surface of the mammalian cell, and thereby identifying compounds which interact with, and specifically bind to, the human glycine transporter.

68. The method of claim 67, wherein the mammalian cell is a non-neuronal cell.

69. The method of claim 68, wherein the non-neuronal cell is a COS7 cell.

70. A method for identifying a compound which is not known to be capable of binding to the human glycine transporter on the surface of a mammalian cell can bind or prevent the binding of a ligand which does so, which comprises contacting the mammalian cell which cell comprises a plasmid adapted for expression in the mammalian cell such plasmid further comprising DNA which expresses the human glycine transporter on the cell surface of the mammalian cell with the compound, determining whether the compound binds to the human glycine transporter or prevents the binding of a ligand which does so, and thereby identifying the compound

as a compound which interacts with, and binds to the human glycine transporter or prevents binding to the glycine receptor by a ligand which does so.

71. The method of claim 70, wherein the mammalian cell is a non-neuronal cell.

72. A pharmaceutical composition comprising a drug identified by the method of claim 67 and a pharmaceutically acceptable carrier.

73. A method of detecting expression of a cell-surface glycine transporter which comprises obtaining total mRNA from the cell, contacting the mRNA so obtained with the nucleic acid probe of claim 20 under hybridizing conditions, detecting the presence of any mRNA hybridized to the probe, the presence of mRNA hybridized to the probe indicating expression of the cell-surface glycine transporter and thereby detecting the expression of the glycine transporter by the cell.

74. A method of treating abnormalities in a subject, wherein the abnormality is alleviated by the reduced expression of a glycine transporter which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 51, effective to reduce expression of the glycine transporter in the subject.

75. A method of treating an abnormal condition related to an excess of glycine transporter activity which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 51, effective to reduce expression of the glycine

transporter in the subject.

76. The method of claim 75, wherein the abnormal condition is epilepsy.

77. The method of claim 75, wherein the abnormal condition is myoclonus.

78. The method of claim 75, wherein the abnormal condition is spastic paralysis.

79. The method of claim 75, wherein the abnormal condition is muscle spasm.

80. The method of claim 75, wherein the abnormal condition is schizophrenia.

81. The method of claim 75, wherein the abnormal condition is cognitive impairment.

82. A method of treating abnormalities which are alleviated by reduction of expression of a mammalian glycine transporter which comprises administering to a subject an amount of the pharmaceutical composition of claim 55 effective to block binding of naturally occurring substrates to the glycine transporter and thereby alleviate abnormalities resulting from overexpression of a mammalian glycine transporter.

83. A method of treating an abnormal condition related to an excess of glycine transporter activity which comprises administering to a subject an amount of the pharmaceutical composition of claim 55 effective to block binding of naturally occurring substrates

to the glycine transporter and thereby alleviate the abnormal condition.

84. The method of claim 83, wherein the abnormal condition is epilepsy.

85. The method of claim 83, wherein the abnormal condition is myoclonus.

86. The method of claim 83, wherein the abnormal condition is spastic paralysis.

87. The method of claim 83, wherein the abnormal condition is muscle spasm.

88. The method of claim 83, wherein the abnormal condition is schizophrenia.

89. The method of claim 83, wherein the abnormal condition is cognitive impairment.

90. A method of detecting the presence of a mammalian glycine transporter on the surface of a cell which comprises contacting the cell with the antibody of claim 43 under conditions permitting binding of the antibody to the transporter, detecting the presence of any antibody bound to the cell, and thereby detecting the presence of a mammalian glycine transporter on the surface of the cell.

91. A method for identifying a substance capable of alleviating the abnormalities resulting from overexpression of a mammalian glycine transporter comprising administering a substance to the transgenic nonhuman mammal of claim 56 and

determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of overexpression of a mammalian glycine transporter.

92. A method for treating the abnormalities resulting from overexpression of a mammalian glycine transporter which comprises administering to a subject an amount of the pharmaceutical composition of claim 49 effective to alleviate the abnormalities resulting from overexpression of a mammalian glycine transporter.

93. A method for identifying a substance capable of alleviating the abnormalities resulting from underexpression of a mammalian glycine transporter comprising administering the substance to the transgenic nonhuman mammal of either of claims 57 or 58 and determining whether the substance alleviates the physical and behavioral abnormalities displayed by the transgenic nonhuman mammal as a result of underexpression of a mammalian glycine transporter.

94. A method for treating the abnormalities resulting from underexpression of a mammalian glycine transporter which comprises administering to a subject an amount of the pharmaceutical composition of claim 50 effective to alleviate the abnormalities resulting from underexpression of a mammalian glycine transporter.

95. A method for diagnosing a predisposition to a disorder associated with the expression of a specific mammalian glycine transporter allele which comprises:

a. obtaining DNA of subjects suffering from the disorder;

b. performing a restriction digest of the DNA with a panel of restriction enzymes;

c. electrophoretically separating the resulting DNA fragments on a sizing gel;

d. contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to DNA encoding a mammalian glycine transporter and labelled with a detectable marker;

e. detecting labelled bands which have hybridized to the DNA encoding a mammalian glycine transporter labelled with a detectable marker to create a unique band pattern specific to the DNA of subjects suffering from the disorder;

f. preparing DNA obtained for diagnosis by steps a-e; and

g. comparing the unique band pattern specific to the DNA of subjects suffering from the disorder from step e and the DNA obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

96. The method of claim 95, wherein a disorder associated with the expression of a specific mammalian glycine transporter allele is diagnosed.

FIGURE 1A

1/21 FIGURE 1A
2/21
3/21
4/21
5/21
6/21
7/21

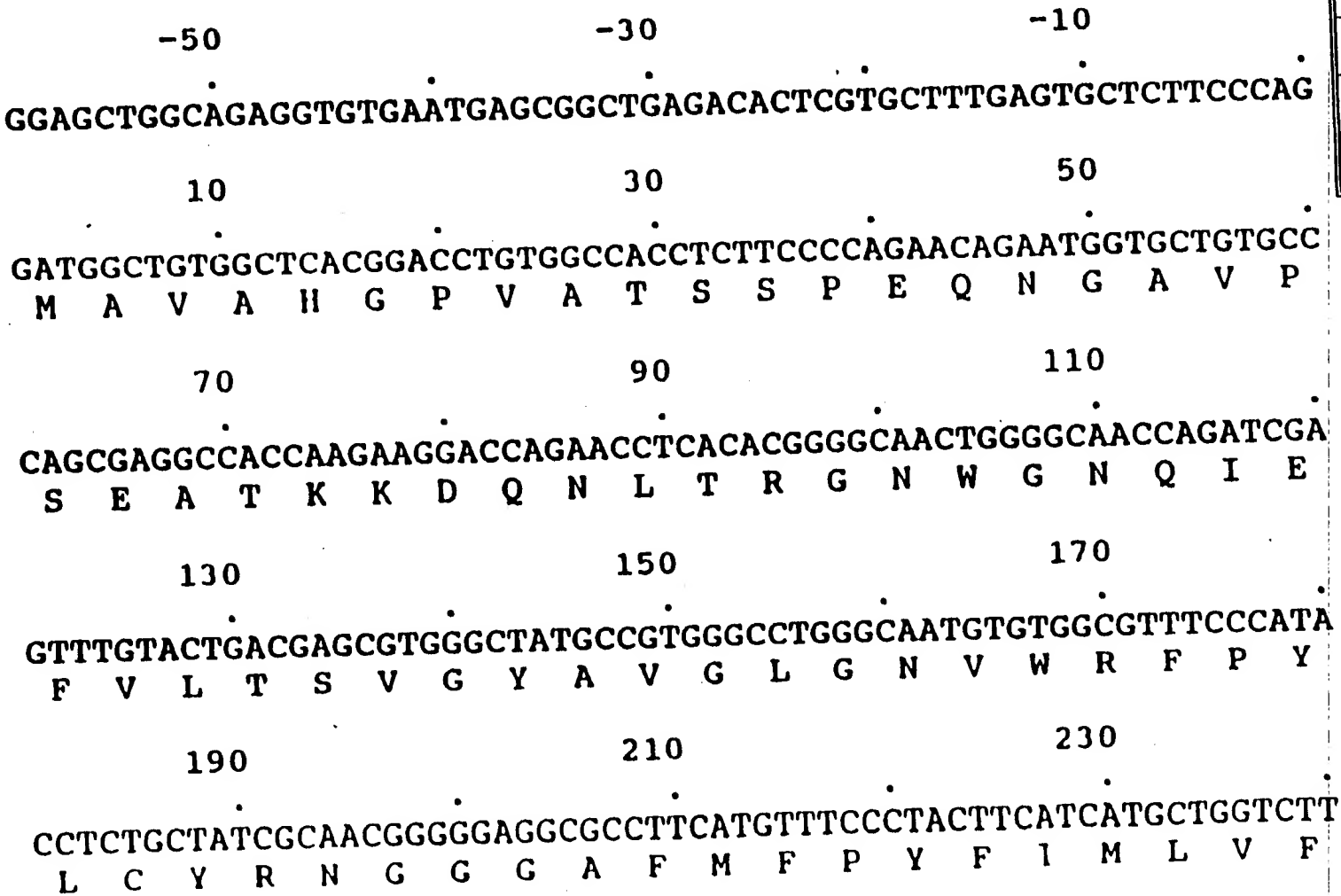

FIGURE 1A CONTINUED

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        250                 270                 290
CTGCGGCATTCCTCTCTTCTTCATGGAGCTCTCCTTCGGCCAGTTTGCAAGCCAGGGCTG
 C  G  I  P  L  F  F  M  E  L  S  F  G  Q  F  A  S  Q  G  C

        310                 330                 350
CCTGGGGGTCTGGAGGATCAGCCCCATGTTCAAAGGCGTGGGCTATGGTATGATGGTGGT
 L  G  V  W  R  I  S  P  M  F  K  G  V  G  Y  G  M  M  V  V

        370                 390                 410
GTCCACGTACATCGGTATCTACTACAACGTGGTCATCTGCATCGCCTTCTACTACTTCTT
 S  T  Y  I  G  I  Y  Y  N  V  V  I  C  I  A  F  Y  Y  F  F

        430                 450                 470
CTCGTCCATGACGCATGTGCTGCCCTGGGCTTACTGCAATAATCCCTGGAACACACCCGA
 S  S  M  T  H  V  L  P  W  A  Y  C  N  N  P  W  N  T  P  D

        490                 510                 530
CTGTGCCGGTGTGCTGGATGCTTCCAATCTCACCAATGGCTCCCGGCCCACTGCCCTGTC
 C  A  G  V  L  D  A  S  N  L  T  N  G  S  R  P  T  A  L  S
```

FIGURE 1A CONTINUED

```
      550                 570                 590
       .                   .                   .                   .
TGGCAACCTGTCTCACCTGTTCAACTACACCTTGCAAAGGACCAGCCCCAGTGAGGAGTA
 G  N  L  S  H  L  F  N  Y  T  L  Q  R  T  S  P  S  E  E  Y

      610                 630                 650
       .                   .                   .                   .
CTGGAGGCTGTATGTGCTGAAGCTGTCGGATGACATTGGAGATTTTGGAGAAGTGCGGCT
 W  R  L  Y  V  L  K  L  S  D  D  I  G  D  F  G  E  V  R  L

      670                 690                 710
       .                   .                   .                   .
TCCTCTCCTAGGCTGCCTTGGCGTCTCCTGGGTGGTTGTCTTCCTCTGCCTCATTCGAGG
 P  L  L  G  C  L  G  V  S  W  V  V  V  F  L  C  L  I  R  G

      730                 750                 770
       .                   .                   .                   .
AGTCAAGTCTTCAGGGAAAGTGGTGTACTTCACGGCCACATTTCCCTATGTGGTGCTGAC
 V  K  S  S  G  K  V  V  Y  F  T  A  T  F  P  Y  V  V  L  T

      790                 810                 830
       .                   .                   .                   .
CATTCTGTTTGTTCGTGGAGTGACCCTGGAAGGAGCCTTCACGGGTATCATGTACTACCT
 I  L  F  V  R  G  V  T  L  E  G  A  F  T  G  I  M  Y  Y  L
```

FIGURE 1A CONTINUED

```
          850                 870                 890
           .                   .                   .                   .                   .                   .
GACCCCAAAGTGGGACAAGATCCTGGAGGCCAAGGTGTGGGGGGATGCAGCCTCTCAGAT
 T  P  K  W  D  K  I  L  E  A  K  V  W  G  D  A  A  S  Q  I

          910                 930                 950
           .                   .                   .                   .                   .                   .
CTTCTATTCCCTGGGCTGTGCATGGGGTGGCCTCATCACCATGGCATCCTACAACAAATT
 F  Y  S  L  G  C  A  W  G  G  L  I  T  M  A  S  Y  N  K  F

          970                 990                 1010
           .                   .                   .                   .                   .                   .
CCACAACAACTGCTACCGGGACAGCGTCATCATCAGCATCACCAATTGTGCTACCAGTGT
 H  N  N  C  Y  R  D  S  V  I  I  S  I  T  N  C  A  T  S  V

          1030                1050                1070
           .                   .                   .                   .                   .                   .
CTATGCTGGCTTCGTCATCTTCTCTATCCTAGGCTTCATGGCCAATCACCTGGGTGTGGA
 Y  A  G  F  V  I  F  S  I  L  G  F  M  A  N  H  L  G  V  D

          1090                1110                1130
           .                   .                   .                   .                   .                   .
TGTGTCTCGGGTGGCAGACCACGGGCCCGGGCTAGCTTTCGTGGCTTACCCCGAGGCTCT
 V  S  R  V  A  D  H  G  P  G  L  A  F  V  A  Y  P  E  A  L
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SUBSTITUTE SHEET

FIGURE 1A CONTINUED

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1150                1170                1190
CACACTGCTTCCCATCTCCCCGCTCTGGTCCTTGCTGTTTTTCTTCATGCTCATCCTGCT
 T  L  L  P  I  S  P  L  W  S  L  L  F  F  F  M  L  I  L  L

1210                1230                1250
GGGACTCGGTACTCAGTTCTGCCTCCTGGAGACCCTAGTCACTGCCATTGTGGATGAGGT
 G  L  G  T  Q  F  C  L  L  E  T  L  V  T  A  I  V  D  E  V

1270                1290                1310
GGGGAATGAGTGGATTCTGCAGAAGAAGACCTACGTGACCTTGGGTGTGGCTGTGGCTGG
 G  N  E  W  I  L  Q  K  K  T  Y  V  T  L  G  V  A  V  A  G

1330                1350                1370
CTTCTTGCTGGGTATCCCTCTTACCAGCCAGGCGGGCATCTACTGGCTGCTGTTGATGGA
 F  L  L  G  I  P  L  T  S  Q  A  G  I  Y  W  L  L  L  M  D

1390                1410                1430
CAACTACGCAGCCAGCTTCTCCTTGGTTGTCATCTCCTGCATCATGTGCGTGTCCATCAT
 N  Y  A  A  S  F  S  L  V  V  I  S  C  I  M  C  V  S  I  M
```

FIGURE 1A CONTINUED

```
      1450                1470                1490
GTATATCTATGGGCACCGGAACTACTTCCAGGACATTCAGATGATGCTGGGGTTCCCACC
 Y  I  Y  G  H  R  N  Y  F  Q  D  I  Q  M  M  L  G  F  P  P

      1510                1530                1550
GCCTCTCTTCTTCCAGATCTGTTGGCGTTTTGTCTCTCCCACTATCATCTTTTTCATTCT
 P  L  F  F  Q  I  C  W  R  F  V  S  P  T  I  I  F  F  I  L

      1570                1590                1610
CATCTTCACGGTGATCCAGTACCGGCCAATCACTTACAACCACTACCAGTACCCAGGCTG
 I  F  T  V  I  Q  Y  R  P  I  T  Y  N  H  Y  Q  Y  P  G  W

      1630                1650                1670
GGCTGTGGCCATCGGCTTCCTCATGGCTTTGTCGTCTGTCATCTGCATCCCATTGTACGC
 A  V  A  I  G  F  L  M  A  L  S  S  V  I  C  I  P  L  Y  A

      1690                1710                1730
ATTGTTCCAGCTCTGCCGCACAGATGGGGACACACTTCTTCAGCGTTTGAAAAATGCCAC
 L  F  Q  L  C  R  T  D  G  D  T  L  L  Q  R  L  K  N  A  T
```

FIGURE 1A CONTINUED

```
1750                1770                1790
                    .                   .                   .
AAAGCCAAGCAGAGACTGGGGCCCTCCTGCCCTCCTGGAGGCACCGGACTGGGCGCTATGCCCC
  K  P  S  R  D  W  G  P  A  L  L  E  H  R  T  G  R  Y  A  P

1810                1830                1850
                    .                   .                   .
CACTACAACCCCCTCTCCTGAAGATGGGTTTGAGGTTCAGCCACTGCACCCGGACAAGGC
  T  T  P  S  P  E  D  G  F  E  V  Q  P  L  H  P  D  K  A

1870                1890                1910
                    .                   .                   .
CCAGATCCCCATCGTGGGCAGTAACGGCTCCAGCCGCCTCCAGGACTCCCGGATATGAGC
  Q  I  P  I  V  G  S  N  G  S  S  R  L  Q  D  S  R  I  *

1930                1950                1970
                    .                   .                   .
ACAGTTGTTGCAAGGGGAGAAGCCCCACCCAACCCTTGCTCCTACCACAGAGACTGAGGA

1990                2010                2030
                    .                   .                   .
GGTGGTGGACCGGTGTGACTGCCTGCCCCATCATGCCCTGGCCAGGGTGGCTGCTGTCAC

2050                .
                    .
CTTGGCCACCACTGCTCATGT
```

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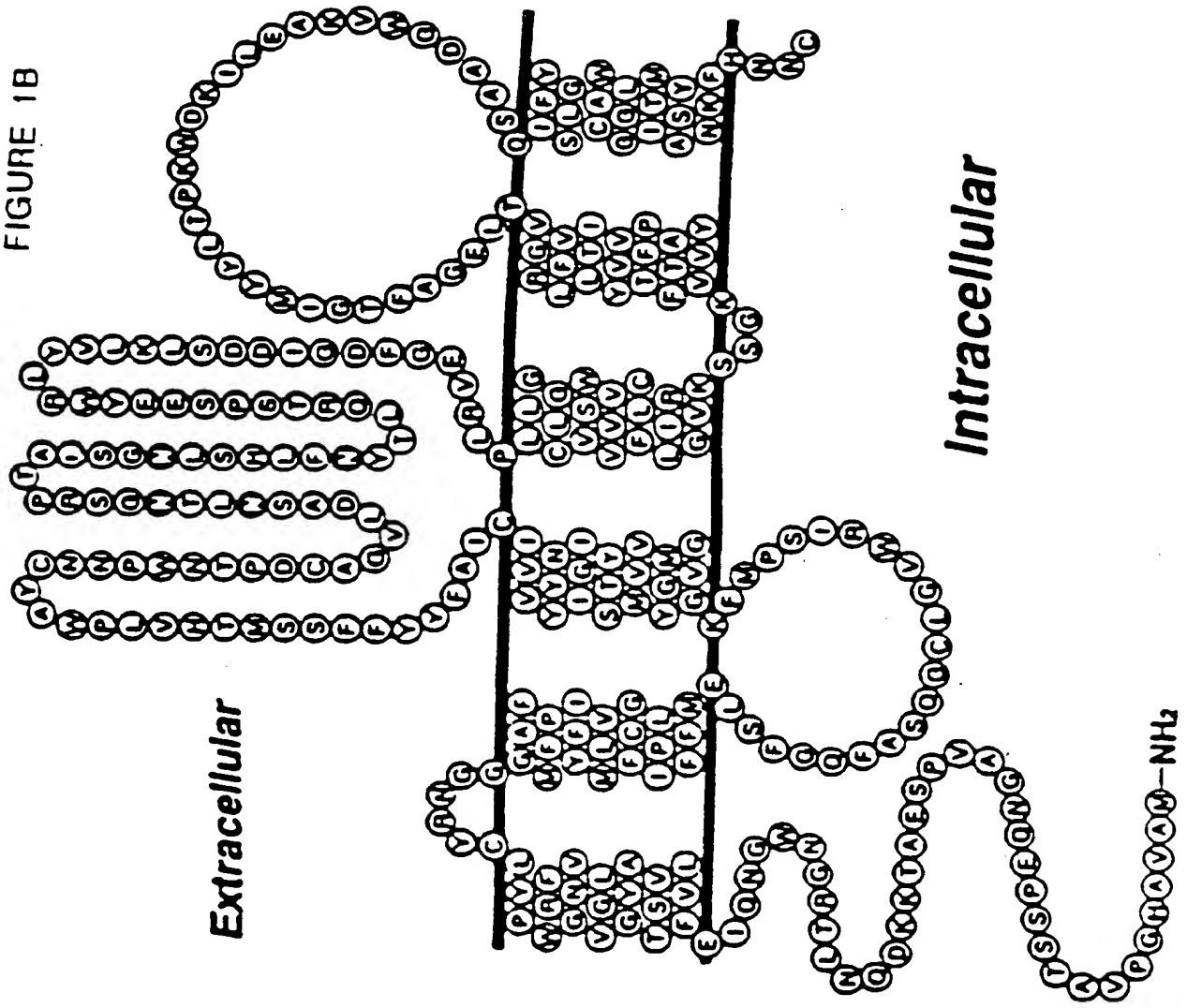
FIGURE 1B



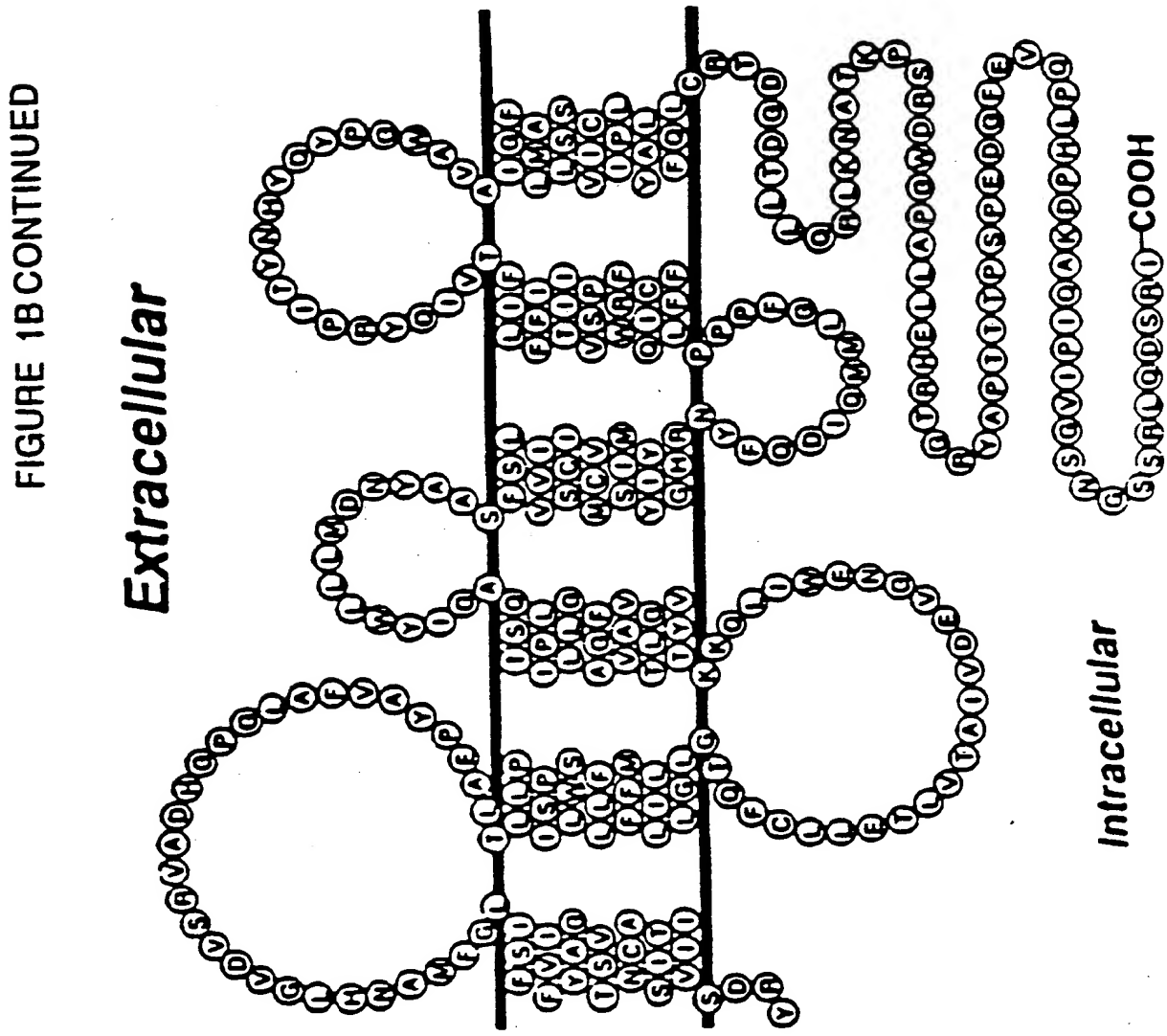


FIGURE 1B CONTINUED

FIGURE 2

```
                                                       ------------- I -------------
Glycine                          MAVAHGPVATSSPEQNGAVPSEATKK   26
   Gaba  MATDNSKVADGQISTEVS.EAPVASDKPKTLVVKVQK.........KA   38
 Norepi MLLARMNPQVQPENNGADTGPEQPLRARKTAELLVVKERNGVQCLLAPRD  50

Glycine DQNLTRGNWGNQIEFVLTSVGYAVGLGNVWRFPYLCYRNGGGAFMFPYFI  76
   Gaba GDLPDRDTWKGRFDFLMSCVGYAIGLGNVWRFPYLCGKNGGGAFLIPYFL  88
 Norepi GDAQPRETWGKKIDFLLSVVGFAVDLANVWRFPYLCYKNGGGAFLIPYTL 100

        ------ II ------                   ------- III -------
Glycine MLVFCGIPLFFMELSFGQFASQGCLGVWRISPMFKGVGYGMMVVSTYIGI 126
   Gaba TLIFAGVPLFLLECSLGQYTSIGGLGVWKLAPMFKGVGLAAAVLSFWLNI 138
 Norepi FLIIAGMPLFYMELALGQYNREGAATVWKICPFFKGVGYAVILIALYVGF 150

Glycine YYNVVICIAFYYFFSSMTHVLPWAYCNNPWNTPDCAGVLDASNLTNGSRP 176
   Gaba YYIVIISWAIYYLYNSFTTTLPWKQCDNPWN....TD....RCFSNYS.. 178
 Norepi YYNVIIAWSLYYLFSSFTLNLPWTDCGHTWNSPNCTD....PKLLNQS.. 194
```

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FIGURE 2
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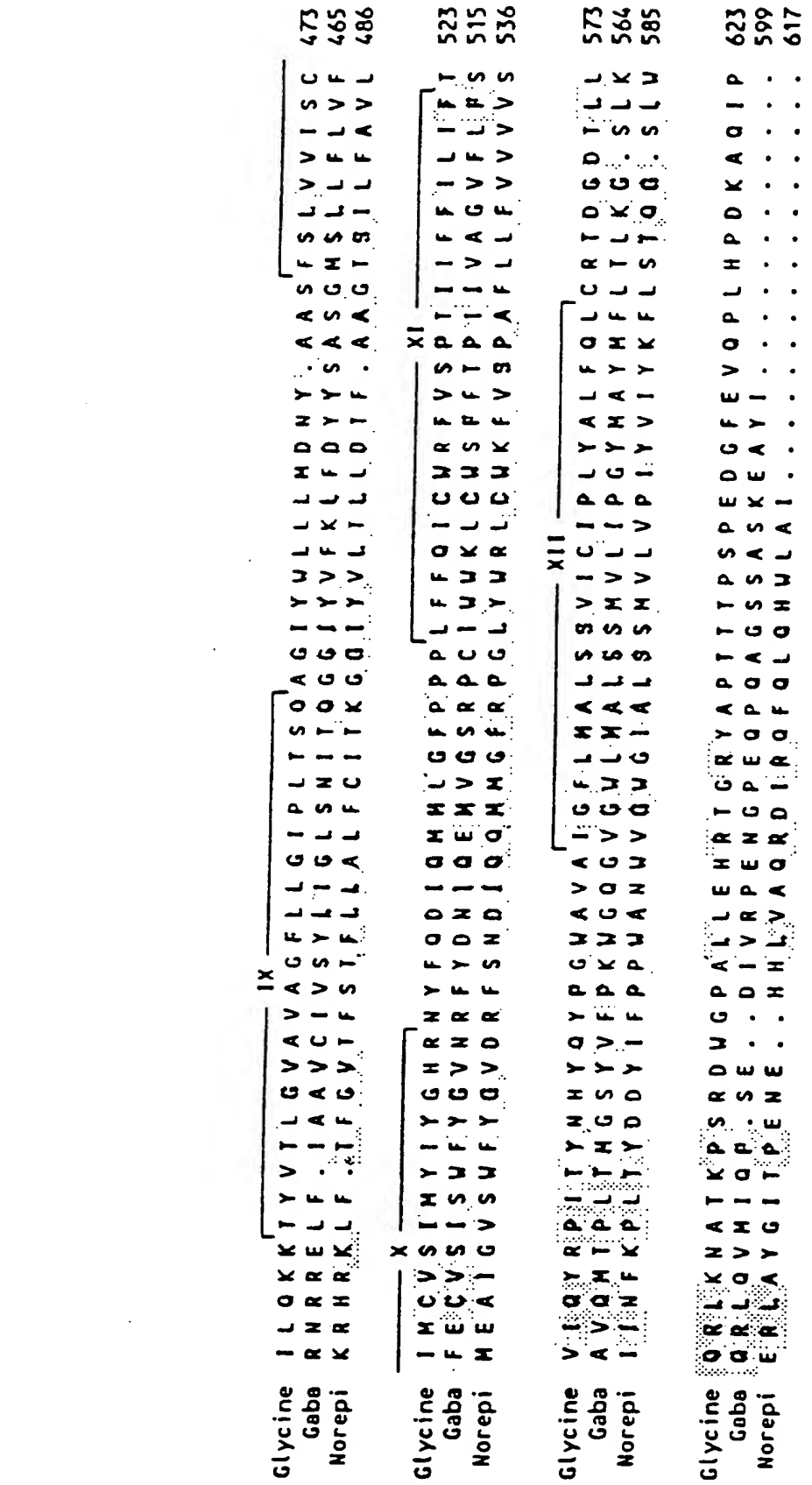

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FIGURE 3

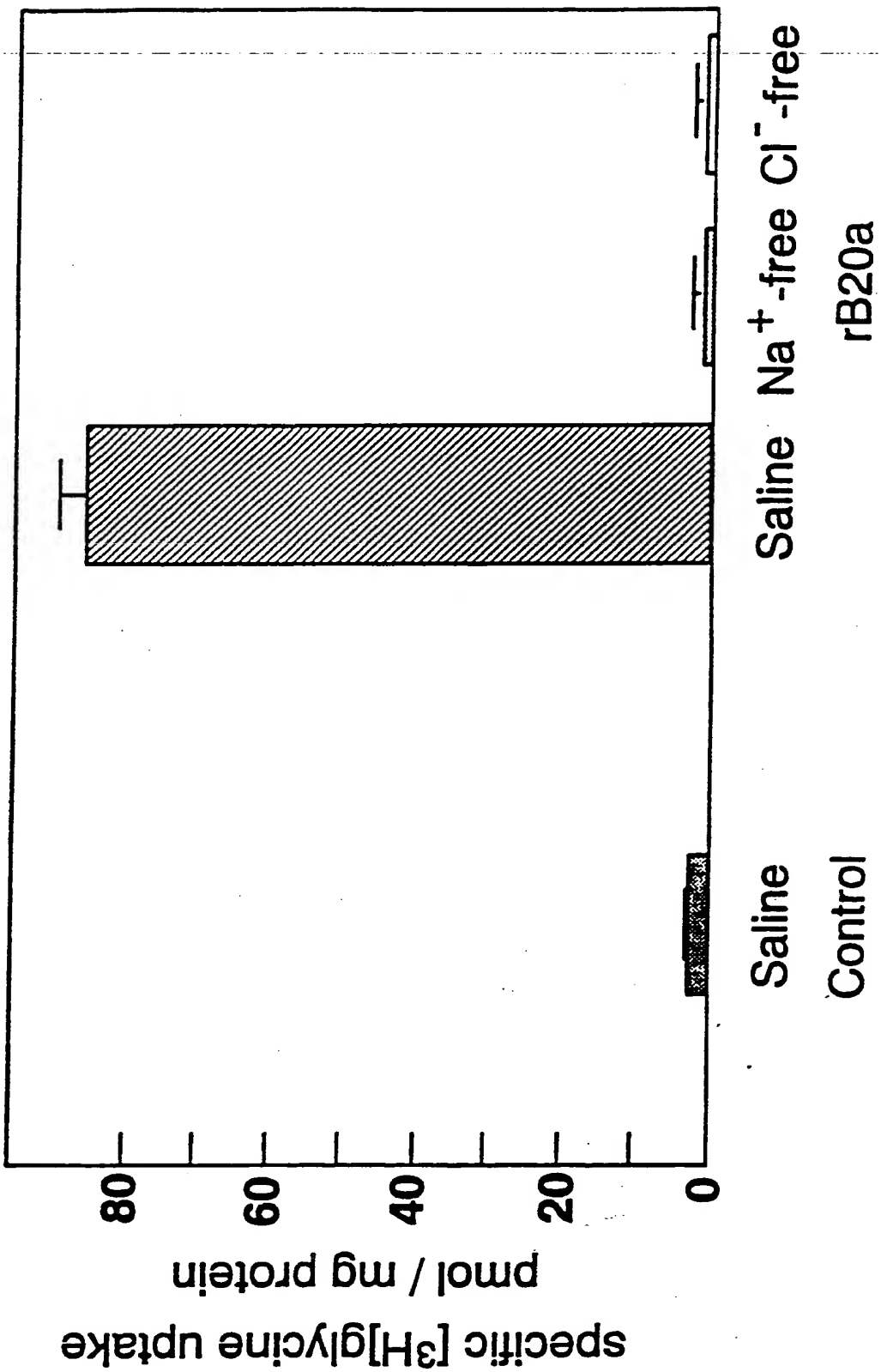

FIGURE 3

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FIGURE 4A

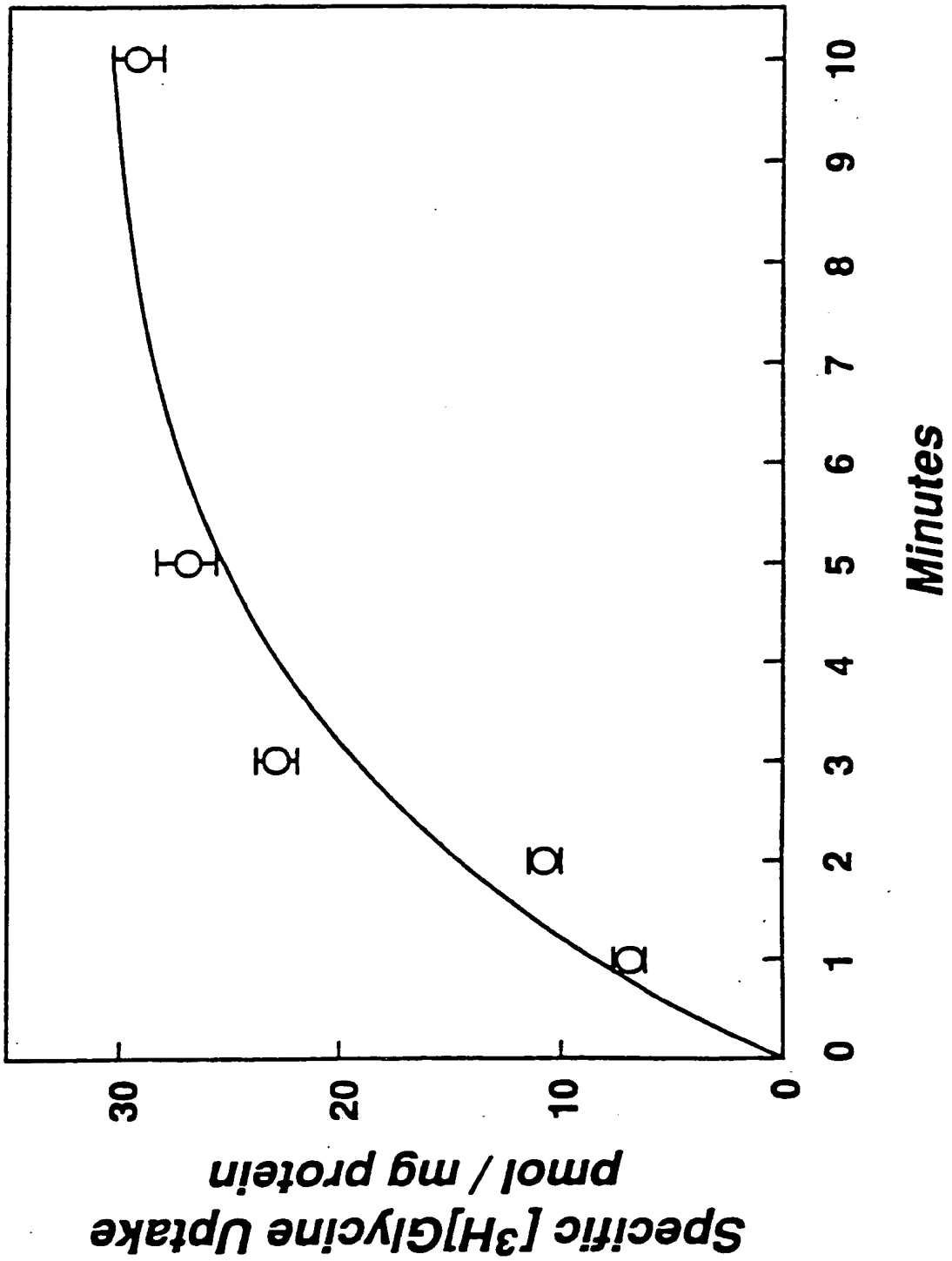

FIGURE 4A

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FIGURE 4B

FIGURE 4B

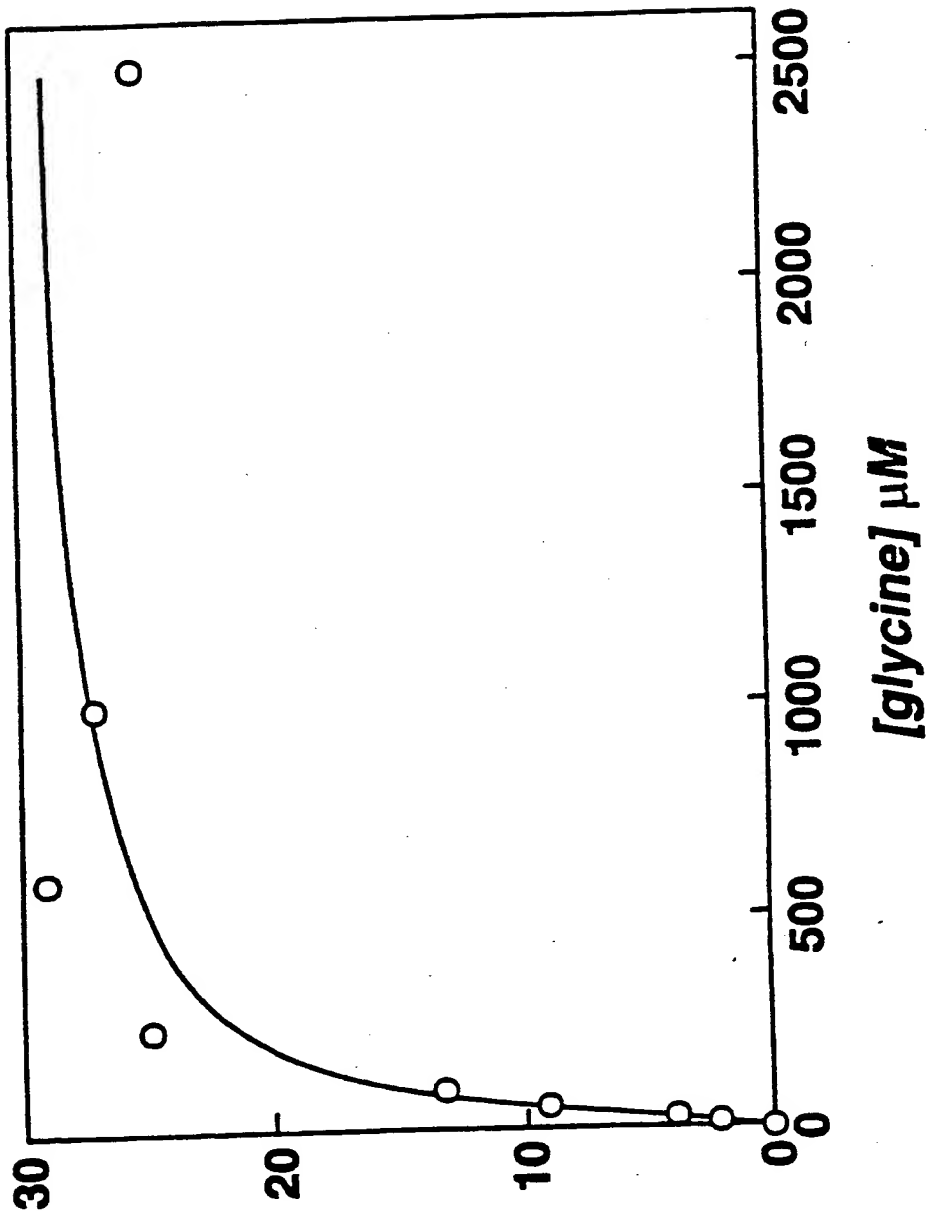


FIGURE 5

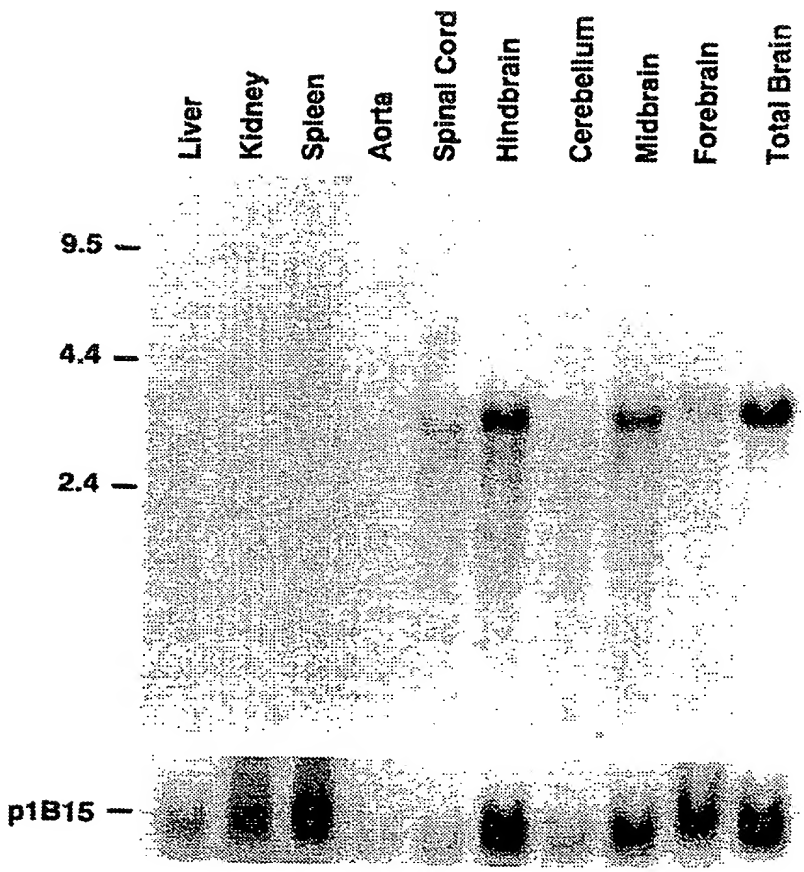


FIGURE 6A

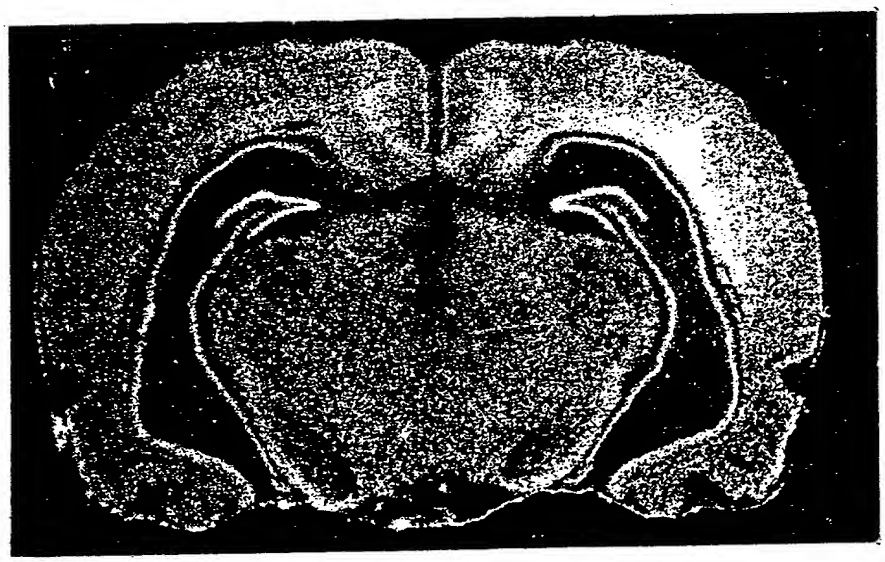


FIGURE 6B

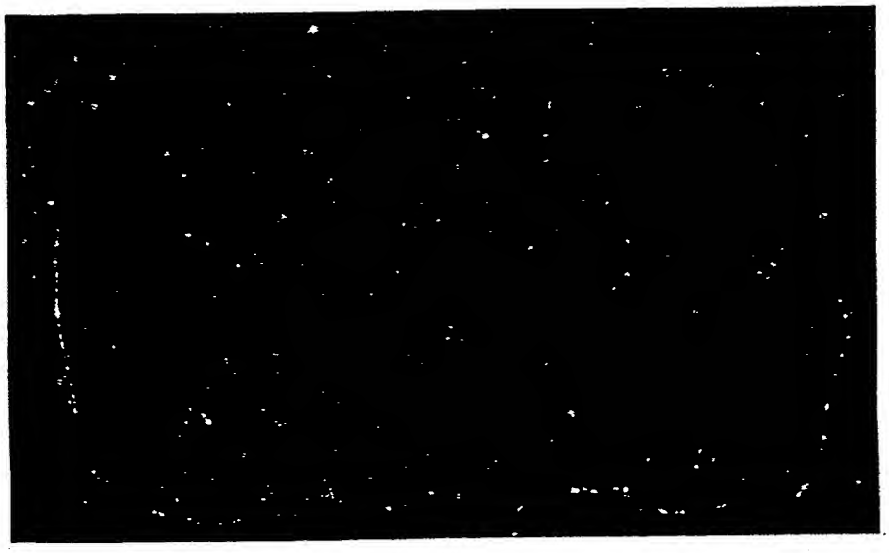
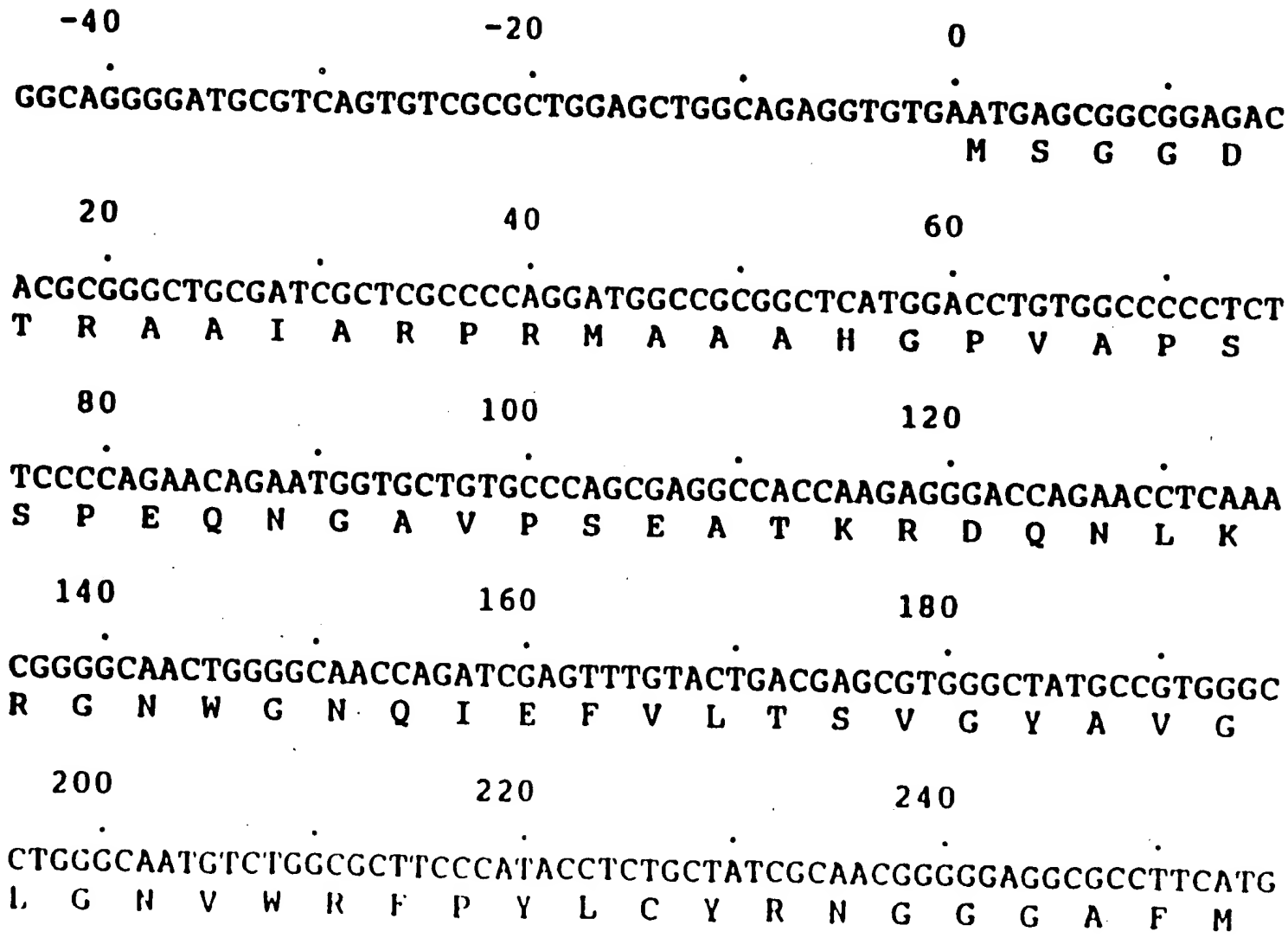


FIGURE 7



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SUBSTITUTE SHEET

FIGURE 7 CONTINUED

```
   260                 280                 300
    .         .         .         .         .         .
TTCCCCTACTTCATCATGCTCATCTTCTGCGGGATCCCCCTCTTCTTCATGGAGCTCTCC
F  P  Y  F  I  M  L  I  F  C  G  I  P  L  F  F  M  E  L  S

   320                 340                 360
    .         .         .         .         .         .
TTCGGCCAGTTTGCAAGCCAGGGGTGCCTGGGGGTCTGGAGGATCAGCCCCATGTTCAAA
F  G  Q  F  A  S  Q  G  C  L  G  V  W  R  I  S  P  M  F  K

   380                 400                 420
    .         .         .         .         .         .
GGAGTGGGCTATGGTATGATGGTGGTGTCCACCTACATCGGCATCTACTACAATGTGGTC
G  V  G  Y  G  M  M  V  V  S  T  Y  I  G  I  Y  Y  N  V  V

   440                 460                 480
    .         .         .         .         .         .
ATCTGCATCGCCTTCTACTACTTCTTCTCGTCCATGACGCACGTGCTGCCCTGGGCCTAC
I  C  I  A  F  Y  Y  F  F  S  S  M  T  H  V  L  P  W  A  Y

   500                 520                 540
    .         .         .         .         .         .
TGCAATAACCCCTGGAACACGCATGACTGCGCCGGTGTACTGGACGCCTCCAACCTCACC
C  N  N  P  W  N  T  H  D  C  A  G  V  L  D  A  S  N  L  T
```

FIGURE 7 CONTINUED

```
                560                 580                 600
                 .                   .                   .
AATGGCTCTCGGCCAGCCGCCTTGCCCAGCAACCTCTCCCACCTGCTCAACCACACAGCCTC
N  G  S  R  P  A  A  L  P  S  N  L  S  H  L  L  L  N  H  S  L

                620                 640                 660
                 .                   .                   .
CAGAGGACCAGCCCCCAGCGAGGAGTACTGGAGGCTGTACGTGCTGAAGCTGTCAGATGAC
Q  R  T  S  P  S  E  E  Y  W  R  L  Y  V  L  K  L  S  D  D

                680                 700                 720
                 .                   .                   .
ATTGGGAACTTTGGGGAGGTGCGGCTGCCCCTCCTTGGCTGCCTCGGTGTCTCCTGGTTG
I  G  N  F  G  E  V  R  L  P  L  L  G  C  L  G  V  S  W  L

                740                 760                 780
                 .                   .                   .
GTCGTCTTCCTCTGCCTCATCCGAGGGGGTCAAGTCTTCAGGGAAAGTGGTGTACTTCACG
V  V  F  L  C  L  I  R  G  V  K  S  S  G  K  V  V  Y  F  T
```

FIGURE 7 CONTINUED

```
800                 820                 840
.         .         .         .         .         .
GCCACGTTCCCCTACGTGGTGCTGACCATTCTGTTTGTCCGCGGAGTGACCCTGGAGGGA
A  T  F  P  Y  V  V  L  T  I  L  F  V  R  G  V  T  L  E  G

860                 880                 900
.         .         .         .         .         .
GCCTTTGACGGCATCATGTACTACCTAACCCCGCAGTGGGACAAGATCCTGGAGGCCAAG
A  F  D  G  I  M  Y  Y  L  T  P  Q  W  D  K  I  L  E  A  K

920
.         .
GTGTGGGGTGATGCTGCCTCC
V  W  G  D  A  A  S
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/09662

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12N 15/00, 15/12; A61K 37/02
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 69.1, 240.2, 320.1; 530/350, 387.1; 514/2, 12; 536/23.1, 23.5, 24.3, 24.31; 800/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG (files 5,155,351,357,358), search terms: glycine, channel, carrier, transporter, receptor, uptake, neurotransmitter

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, Volume 89, issued August 1992, J. Guastella et al., "Cloning, expression, and localization of a rat brain high-affinity glycine transporter," pages 7189-7193, see entire document.	1, 35-37, 39 2-5, 9-27, 28-34, 38, 40-96
X,P Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, Volume 89, issued July 1992, Q.-R. Liu et al., "A family of genes encoding neurotransmitter transporters," pages 6639-43, see entire document.	6-8 1-5, 9-27, 32, 34-36
X,P Y	FEBS LETTERS, Volume 305, Number 2, issued June 1992, Q.-R. Liu et al., "Cloning and expression of a glycine transporter from mouse brain," pages 110-114, see entire document.	1, 6-8, 35-37, 39 2-5, 9-27, 28-34, 38, 40-96
X,P Y	FEBS LETTERS, Volume 295, Number 1,2,3, issued December 1991, W. Mayser et al., "Isolation of cDNAs encoding a novel member of the neurotransmitter transporter gene family," pages 203-206, see entire document.	6-8 1-5, 9-96
Y	WO, A, 90/06047 (LAM ET AL) 14 June 1990, see pages 5-6 and Example III at page 29.	1-96

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
26 January 1993

Date of mailing of the international search report
08FEB 1993

Name and mailing address of the ISA/
Commissioner of Patents and Trademarks
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MARIANNE PORTA ALLEN
Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/09662

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SCIENCE, Volume 254, issued 25 October 1991, B.J. Hoffman et al., "Cloning of a Serotonin Transporter Affected by Antidepressants," pages 579-580, see entire document.	1-27, 32, 34-36
X	NATURE, Volume 354, issued 07 November 1991, R.D. Blakely, "Cloning and expression of a functional serotonin transporter from rat brain," pages 66-70, see entire document.	6-8
Y		1-5, 9-27, 32, 34-36
Y	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, Volume 167, Number 1, issued 28 February 1990, Z.-Y. Zhao et al., "Isolation of Distantly Related Members in a Multigene Family Using the Polymerase Chain Reaction Technique," pages 174-182, see entire document.	1-27, 32, 34-36
Y	G. SCANGOS et al., "ADVANCES IN GENETICS," Volume 24, published 1987 by ACADEMIC PRESS, INC. (N.Y.), pages 285-322, see pages 285-322.	56-61

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/09662

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/6, 7.1, 69.1, 240.2, 320.1; 530/350, 387.1; 514/2, 12; 536/23.1, 23.5, 24.3, 24.31; 800/2

Form PCT/ISA/210 (extra sheet)(July 1992)*